Mycobacteria and zoonoses among pastoralists and their livestock in South-East Ethiopia

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2. Summary

Globally, tuberculosis (TB) causes millions of deaths per year. Ethiopia ranks seventh among the world's 22 countries with high tuberculosis burden. *Mycobacterium tuberculosis (M. tuberculosis)* is the most common cause of human TB, but an unknown proportion of cases are due to *M. bovis*. Although cattle are considered to be the main hosts of *M. bovis*, isolations have been made from many other livestock and wildlife species and transmission to humans constitutes a public health problem. BTB became rare in human and cattle in developed countries as the result of milk pasteurization and test and slaughter policy. A Test and slaughter control option is difficult to apply in developing countries due to high cost of implementation. TB caused by *M. bovis* is clinically indistinguishable from TB caused by *M. tuberculosis* and can only be differentiated by laboratory methods. A considerable amount of suspected human TB and TB like-lesions specimens in animals were identified as non-tuberculous mycobacteria (NTM) in numerous reports. Conventional diagnostic laboratory methods are not accurate enough to identify NTM from *Mycobacterium tuberculosis* complex (MTC). Polymerase chain reaction (PCR) based molecular techniques are appropriate methods to differentiate NTM from MTC.

BTB is endemic in Ethiopian cattle in central highlands and the situation is not well known in pastoral areas. Further more a zoonotic link of *M. bovis* was not documented in Ethiopia. Limited information is available on the status of brucellosis and Q-fever in livestock of southeast Ethiopian pastoralists. In the present study we investigate the presence of zoonotic transmission of tuberculosis at the human-livestock interface and assess the status of brucellosis and Q-fever in pastoral livestock of southeast Ethiopia in Oromia and Somali Regional States.

Comparative intradermal tuberculin test (CIDT) was conducted in 894 cattle from Dhuko, Sirba, Arda-Bururi and Siminto pastoral association (PA) in Oromia and Hayadimtu, Bifatu, Melkalibe and Bakaka PAs in Somali region. In addition 479 camels and 518 goats were included from the same PAs in Somali region. The test results were interpreted based on the Office Internationale des Epizooties (OIE) recommended 4 mm and a recently suggested 2 mm cut-off for the CIDT test in cattle and >4 mm was used for camels and goats. The individual animal prevalence of tuberculin reactors was 4.0% (95% CI= 2.7-5.3%) and 5.4% (95% CI= 3.9-6.8%) when using the 4 mm and the 2 mm cut-off,

respectively. BTB prevalence was 0.4% (95% CI= -0.2-1.0%) and 0.2 %(95% CI=-0.2-0.6%) in camels and goats, respectively. In Somali region prevalence of avian PPD reactors in cattle, camels and goats were 0.7% (95% CI= 0.2-2.0%), 10.0 %(95% CI= 7.0-14.0 %) and 1.0% (95% CI= 0.3-4.0%), respectively, whereby camels had an odds ratio (OR) of 16.5 (95% CI=5.0- 55.0) when compared to cattle. As compared to Somali regions high prevalence of BTB reactor cattle were from Oromia region with some hot spot PAs; Arda-Bururi and Siminto in Oromia and Hayadimitu in Somali region and risk factors to be further investigated. The high proportion of camel reactors to avian PPD needs further investigation of its impact on camel production.

Sputum and fine-needle aspirate (FNA) specimens were collected from 260 patients with suspected pulmonary TB and from 32 cases with suspect TB lymphadenitis, respectively. In parallel, 207 suspected tuberculous lesions were collected from livestock slaughtered at abattoirs. Specimens were processed and cultured for mycobacteria; samples with acid-fast stained bacilli were further characterized by molecular methods including genus and deletion typing as well as spoligotyping. Non-tuberculous mycobacteria (NTM) were sequenced at the 16S rDNA locus. Culturing and molecular typing of acid-fast bacilli collected from humans yielded 174 (67%) and 9 (28%) mycobacterial isolates from sputum and FNA, respectively, of which 161 were characterized as *M. tuberculosis*, three were *M. bovis*, and the remaining 10 were typed as NTMs. Similarly, a yield of 40 (23%) mycobacterial isolates was recorded from tuberculous lesions of livestock animals, including 24 *M. bovis* and 4 NTMs from cattle, 1 *M. tuberculosis* and 1 NTM from camels, and nine NTMs from goats. Isolation of *M. bovis* from humans and *M. tuberculosis* from animal confirmed transmission between livestock and humans in the pastoral areas of southeast Ethiopia.

Simultaneous surveys of brucellosis and Q-fever were conducted in animals tested for tuberculin skin test. Sera were collected from all livestock tested for BTB to assess the status of brucellosis and Q-fever in pastoral livestock of study area. A total of 1830 animals comprising 862 cattle, 458 camels and 510 goats were screened initially with Rose Bengal test (RBT) for brucellosis. All RBT positive and 25% of negative animals were further tested using ELISA. These comprise a total of 460 animals (211 cattle, 102 camels and 147 goats). Besides, sera from a total of 368 animals (180 cattle, 90 camels and 98 goats) were

tested for Q-fever using ELISA kit. The sero-prevalence of brucellosis in RBT tested animals was 1.4% (95% CI= 0.8, 2.6%), 0.9% (95% CI= 0.3, 2.7%) and 9.6% (95% CI =5.2, 17.1) in cattle, camels and goats, respectively. Twelve percent (12.0%) of negative camel sera were positive for ELISA. Thus, ELISA is more sensitive than RBT in the present study. The sero-prevalences of Q-fever were 31.6% (95% CI=24.7-39.5%), 90.0% (95% CI= 81.8-94.7%) and 54.2% (95% CI= 46.1-62.1%) in cattle, camels and goats, respectively. Both brucellosis and Q-fever are prevalent in the study area. High seropositivity of Q-fever in all livestock species tested and higher seropositive in goats for brucellosis implies risks of human infection by both diseases. Thus, warrant further study of both diseases in animals and humans in the area.

The simultaneous study of mycobacteria in humans and livestock, and other zoonoses in the present study demonstrates an added value of a "One Health" approach of closer cooperation of human and animal health sectors in Ethiopian pastoralists

3. Summary in Amharic

የሳንባነቀርሳ (ቲቢ) በሽታ በየዓመቱ በሚሊዮን የሚቆጠሩ ዜጎችን ይቀጥፋል፡፡ በከፍተኛ ደረጃ በቲቢ በሽታ ከሚጠቁ ከ22ቱ የዓለም ሀገሮች መሃል ኢትዮጲያ በሰባተኛ ደረጃ ላይ ትገኛለች፡፡ በሰው የቲቢ በሽታ መንስኤ ከሆኑ ተዋህሲያን በቀደምትነት የሚጠቀሰው ***ማይኮባክተሪየም ቱበርኩሎሲስ*** ባክተሪያ ሲሆን ከዳልጋ ከብት ወደ ሰው የሚተላለፈው ደግሞ ***ማይኮባክተሪየም ቦቪስ*** የተባለው ነው፡፡ ***ማይኮባክተሪየም ቦቪስ*** በዋናነት የከብት በሽታ መሆኑ የታወቀ ቢሆንም በሽታው የተለያዩ የቤትና የዱር እንስሳትንም እንደሚያጠቃ ይታወቃል፡፡ በሽታው ከእንስሳ ወደ ሰው ሲተላለፍ በህብረተሰብ ላይ የጤና ችግር ከማስከተል ባሻገር በከፍተኛ ላብራቶሪ ምርመራ ካልሆነ በስተቀር ከሰው ቲቢ መንስኤ ከሆነው መለየት አይቻልም፡፡ ባደጉት ሀገሮች የወተት ፓስቸራይዘሽንና እንስሳትን መርምሮ የማስወገድ የበሽታ መቆጣጠሪያ ዘዴ በመጠቀማቸው የዳልጋ ከብት ቲቢ ክስተት በጣም አነስተኛ ሆኗል፡፡ ከላይ በተጠቀሰው የመቆጣጠሪያ ዘዴ በሽታውን ለመከላከል ወጪው ከፍተኛ ስለሆነ በታዳጊ ሀገሮች የሚቻል አይደለም ፡፡

የዳልጋ ከብት ቲቢ በሽታ በመሃል ኢትዮጲያ ተሰራጭቶ እንደሚገኝ የሚታውቅ ሲሆን ሁኔታው በአርብቶ አደሮች አካባቢ በውል አይታወቅም፡፡ በተጨማሪም ከእንስሳ ወደሰው ከመተላለፉ ጋር ተያይዞ የተመዘገቡ መረጃዎች የሉም፡፡ ከቲቢ በሽታ በተጨማሪም ከእንስሳ ወደ ሰው የሚተላለፉ በርካታ በሽታዎች መኖራቸው የሚታወቅ ሲሆን ከነዚህም መሃል እንደነ ***ብሩሰሎሲስ*** እና ***ክውፊቨር*** ያሉትን ለአብነት መጥቀስ ይቻላል፡፡ ስለእነዚህ በሽታዎች በደቡብ ምሥራቅ ኢትዮጲያ አርብቶ አደሮች አካባቢም ያሉ መረጃዎች ውስን በመሆናቸው እነዚህ በሽታዎች ያሉበት የስርጭት ሁኔታ በውል አይታወቅም፡፡

ከላይ የተጠቀሱ ሁኔታዎችን ከግምት ውስጥ በማስገባት ባለፉት ሶስት ዓመታት እንደ አውሮፓውያን አቆጣጠር ከ2008 እስከ 2010 ባለው ጊዜ ውስጥ በእንስሳትና በሰው ቲቢ እንዲሁም በ***ብሩሰሎሲስ*** እና በ***ክውፊቨር*** በሽታዎች ላይ በደቡብ ምሥራቅ ኦሮሚያና በሶማሌ ክልላዊ መንግስት አርብቶ አደሮች አካባቢ ጥናት ተካሂዶ ነበር፡፡ ጥናቱ በቅንጅትና በርካታ ባለሙያዎችን ያካተተ ሲሆን በቀደምትነት የሚጠቀሱ የእንስሳትና የሰው ህክምና ባለሙያዎች ቡድን የተሳተፈበት ነበር፡፡

የጥናቱ ዓላማዎችም ሀ) በደቡብ ምስራቅ ኢትዮጲያ አርብቶ አደሮች አካባቢ በማይኮባክተሪያ የሚተላለፉ በሽታዎች ሥርጭትን በሰውና በእንስሳት ያለበትን ሁኔታ

ማወቅ ለ) የዳልጋ ከብቶች ቲቢ በአካባቢው የቲቢ ሥርጭት ያለው ድርሻ ምንያህል እንደሆነ ማወቅ ሐ) በአካባቢው ተሰራጭቶ የሚገኘውን የሰውና የዳልጋ ከብቶች የቲቢ ዝርያዎችን በሞለኩላር የምርመራ ዘዴ ለይቶ ማወቅ መ) ከእንስሳ ወደ ሰው የሚተላለፉ እንደ *ብሩሰሎሲስ* እና *ክውፊቨር* ያሉትን ሌሎች በሽታዎች ማጥናት ናቸው።

በዚሁ መሠረት በመስክና በላብራቶሪ የቲቢና ሌሎች ከእንስሳ ወደ ሰው በሚተላለፉ በሽታዎች ላይ ጥናት ተካሂዷል። የመስክ ጥናት የተካሄደው በኦሮሚያ ክልል በዱቆ፣ሲርባ፣አርዳቡሩሪና የሲሚንቶ አርብቶ አደሮች ሲሆኑ በሶማሌ ክልል በሃያድምቱ፣ ብፋቱ፣ መልካሊበ እና በቀቃ አርብቶ አደር መንደሮች ናቸው። በመስክ ጥናት የተካተቱ የቤት እንስሳትም 894 ከብቶች፣ 497 ግመሎች እና 518 ፍየሎች ነበሩ። ከተመረመሩ እንስሳት መሃል 4.0% ከብቶች ፣ 0.4% ግመሎች እና 0.2% ፍየሎች የዳልጋ ከብት ቲቢ በሽታ መጋለጣቸውን የምርመራ ውጤት ያሳያል። ከተመረመሩት ግመሎች መሃል 10.0%ቱ ወፎችን ከሚያጠቃው የማይኮባክተሪያ ዝርያ መጋለጣቸውን ያሳያል። ከሶማሌ ክልል ጋር ሲነፃፀር የዳልጋ ከብት ቲቢ ስርጭት በኦሮሚያ ክልል በርከት ያለ ስሆን አንዳንድ ቦታዎች ላይ ስርጭቱ ከተጠበቀው በላይ ሆኖ ታይቷል። በኦሮሚያ አርዳቡሩሪ እና ሲሚንቶ በሶማሌ በሃያድምቱ አርብቶ አደሮች መንደር ከፍተኛ የበሽታ ስርጭት ከታየባቸው መሃል ለአብነት መጥቀስ ይቻላል። ለእነዚህ ምክንያቶች ሊሆኑ የሚችሉ መንስኤዎችን ለማወቅ ለወደፊት በጥልቀት ጥናት ማካሄድ ያስፈልጋል። ከሌሎች እንስሳት በተለየ ሁኔታ ግመሎች ለወፎች ዝርያ ማይኮባክተሪያ ስለመጠቃታቸው ሁኔታም በግመል እርባታ ላይ ያለውን ተፅዕኖ ከግምት በማስገባት ለወደፊቱ ጥናት ማድረግ ያስፈልጋል።

በላብራቶሪ ምርመራ በተደረገው ጥናት ከ260 የቲቢ በሽተኞች የተወሰደው የአክታ ናሙና እና 32 ከሰው የአንገት ዕጢ ዕባጭ እንዲሁም 207 ናሙናዎች በአካባቢው ቄራዎች ከሚታረዱ እንስሳት ተወስዶ አዲስ አባባ ከሚገኘው አርማወር ሐንሰን ምርምር ኢንስቲትዩት ጥልቅ ምርመራ ተካሂዶበታል። ከእነዚህ ናሙናዎች መሃል 89% የሰው እና 23% የእንስሳት ናሙናዎች ማይኮባክተሪያ እንደተገኘባቸው ተረጋግጧል።

ሞሎኩላር ምርመራ ከተደረገባቸው ከሰው ናሙናዎች መሃል 92.5%ቱ የሰው ቲቢ፣ 1.9% የዳልጋ ከብት ቲቢ እና 5.8% ቲቢ ካልሆነ ማይኮባክተሪያ ዝርያ መሆኑ ታውቋል። በተመሣሣይ ሁኔታ ከእንስሳት ናሙናዎች መሃል 60% የዳልጋ ከብት ቲቢ 2.5% የሰው ቲቢ እና 25.0% ቲቢ ካልሆኑ ማይኮባክተሪያ ዝርያ ናቸው።

በዚህ ጥናት የዳልጋ ከብት ቲቢ ዝርያ በሰው ላይ መገኘቱ እና የሰው ቲቢም በእንስሳት ላይ መገኘቱ ቲቢ ከእንስሳት ወደ ሰው እንዲሁም ከሰው ወደ እንስሳ የሚተላለፍበት ክስተቶች መኖራቸው የተረጋገጠበት ነበር።

በመስክ የቲቢ በሽታ ምርመራ የተደረገላቸው እንስሳት በሙሉ የደም ናሙናዎች ተወስዶ የ*ብሩሰሎሲስ* እና *ክውፊቨር* በሽታዎች በተጨማሪ የላብራቶሪ ምርመራ ተደርጎ ነበር። በአጠቃላይ 862 ከብቶች፣ 458 ግመሎች እና 510 ፍየሎች በሮዘበንጋል ምርመራ ተደርጎላቸው 1.4% ከብቶች፣ 0.9% ግመሎች እና 9.6% ፍየሎች ለ*ብሩሰሎሲስ* በሽታ መጋለጣቸው ታውቋል።

በሮዝበንጋል ምርመራ ነጌቲቭ ከሆኑት ግመሎች መሃል 12.0%ቱ ለኢላዛ ምርመራ ፖዝቲቭ ሆኖ ተገኝቷል። በዚህ ጥናት የኢላዛ ምርመራ በግመሎች ከሮዝባንጋል የበለጠ የ*ብሩሰሎሲስ* በሽታን መለየት ላለመለየቱ ተጨማሪ የማረጋገጫ ጥናት ያስፈልጋል። ለ*ክውፊቨር* በሽታ የተደረገው ምርመራ 32.0% ከብቶች፣ 54.2% ፍየሎች እና 90.0% ግመሎች ለበሽታ የተጋለጡ መሆናቸውን ያሳያል። ጥናቱ እንደሚጠቁመው ሁለቱም በሽታዎች በአካባቢው ተሠራጭተው እንደምገኙ የሚያሳይ ሲሆን፣ የአካባቢው አርብቶ አደሮች የአኗኗር ሁኔታም ከእንስሳት ጋር ያለው ቅርርብ ከፍተኛ በመሆኑ እና ጥሬ ሥጋ መብላትና ያልተፈላ ወተት መጠጣት በሰፊው የሚዘወተር በመሆኑ እነዚህ በሽታዎች ወደ ሰው የመተላለፍ እድላቸው ከፍተኛ ነው። ስለሆነም ለወደፊቱ የበሽታዎቹ ሁኔታ በሰውና በእንስሳት በተጓዳኝ መጠናት አለበት።

በጥናቱ እንደተመለከተው የሰውና የእንስሳት ጤና ባለሙያዎች በትብብርና በቅንጅት ጥናቱን መሥራታቸው፣እንዲሁም የተለያዩ የበሽታ አይነቶች ጎን ለጎን መጠናታቸው "**የጋራ ጤና**" ጽንሰ ሃሳብን ለማሳደግ አግዟል።

4. Abbreviations

AFB - Acid Fast Bacilli

AHINTC - Avian and Human Influenza National Technical Committee

AHRI- Armauer Hansen Research Institute

BCG - Bacillus Calmette-Guèrin

BTB - bovine tuberculosis

CFSPH - The Center for Food Security and Public Health

CI- Confidence Interval

CIDT - Comparative Intradermal tuberculin Test

CVM - Christian Veterinary Mission

DNA - Deoxyribonucleic Acid

DOTS - Direct Observed Treatment Strategy

EPTB - Extra Pulmonary Tuberculosis

EU1 - European 1

FMOH - Federal Ministry of Health

FNA - Fine Needle Aspirate

GDP- Gross Domestic Product

GEE - Generalized Estimating Equation

HIV- Human Immunodeficiency Virus

I-ELISA- Indirect –Enzyme Linked Immunosorbent Assay

LSP- Large Sequence Polymorphism

MAC- *Mycobacterium avium*-Complex

MOH- Ministry of Health

MTC- Mycobacterium Tuberculosis-Complex

NTM- Nontuberculous Mycobacteria

OIE- Office Internationale des Epizooties

OR- Odds Ratio

PA- Pastoralist Association

PFE- Pastoralist Forum Ethiopia.

PCR- Polymerase chain reaction

PPD- Purified Protein Derivate

RBT- Rose Bengal plate Test

RDAF1- Region of Difference Africa 1

RDAF2- Region of Difference Africa 2

RDEU1- Region of Difference European 1

rDNA - Ribosomal Deoxyribonucleic Acid

RD- Region of Difference

rRNA - Ribosomal Ribonucleic Acid

RT- Rapid Test

SNNP- Southern Nations, Nationalities, and People's Region

SNP- Single Nucleotide Polymorphism

Swiss TPH - Swiss Tropical and Public Health Institute

TB- Tuberculosis

VLA- Veterinary laboratories Agency

WHO- World health organization

ZN- Ziehl Neelson

5. Introduction

5.1. 1. Pastoral systems

Pastoralism refers to a livelihood based on livestock raising, and can be undertaken by sedentary or mobile communities [1]. Pastoralism is found in many forms throughout the world [2, 3]. Composition of herds, management practices, social organization and all other aspects of pastoralism vary between areas and social groups. Traditional pastoral production systems of Africa may be classified (in order of increasing mobility) as agro-pastoralism (sedentary pastoralism), semi-sedentary-pastoralism (transhumance) and nomadic or migratory pastoralism with a high degree of mobility [1]. Mobility allows pastoralists to simultaneously exploit more than one environment, thus creating the possibility for arid regions to support human life. The traditional pastoral systems in arid and semi-arid regions of sub-Saharan Africa used to cope effectively and in an environmentally sustainable manner with the prevailing harsh and erratic ecological conditions of those regions. The ability to move their herds over large distances, grazing the diffuse and scattered vegetation of the regions' rangelands, and being able to take refuge to more favorable sites during droughts, was the foundation of their system, and was critical to their livestock and their own livelihoods[4].

Ethiopian pastoralists are estimated about 13.7% of the total population of the country. They inhabit the arid and semi-arid parts of the country and they have been among economically and politically marginalized populations [5]. They are mainly classified as pastoral and agro-pastoral production system. Pastoralists move the herd seasonally from one area to another for the search of pasture and water, whereas agro-pastoralists are based on mixed farming practices, livestock rearing with limited mobility as compared to pastoralists, and crop cultivation to supplement their livestock production [6,7]. Sixty percent (60%) of the national territory is pastoral and agro-pastoral using area below 1500-m elevation as crude thresholds (Figure 5.1). Out of 11 regional states of Ethiopia seven regions have pastoralist communities. The proportion of pastoral communities in these regions varies and some regions like Somali and Afar are totally inhabited by pastoralist communities whereas other region has varying proportion of pastoralist components. Somali and Oromia regions share large proportions (Table 5.1).

The current regional structure of Ethiopia is based on the ethnic territories and pastoralists in different regions also belong to different ethnic groups. Although they share the common characteristics of mobility and livestock based livelihood, they have substantial inter-regional differences in cultural practices, religions and livestock systems. For example pastoralists in Gambella and Beneshangul Gumuz keep cattle and small ruminants as main livestock whereas in the Oromia region except Karayu, pastoralist who keep camel as main livestock, the majority is cattle and small ruminant keepers. In some areas of Oromia, pastoralists like Guji and Borana they posses cattle as main livestock and additionally camels and small ruminants. The main livestock kept by Somali and Afar pastoralist are camels and small ruminants whereas cattle are an additional asset. These inter-regional differences in different pastoral groups are also reflected in their knowledge of livestock diseases and husbandry practices. For example, Guji and Borana pastoralists are better in the knowledge of cattle and goat diseases than camel, whereas Somali pastoralists are experts in camel husbandry and the identification of their illnesses.

However the pastoralists have common characteristics of raw milk consumption, some have differences in meat consumption habits. Traditional Borana and Somali pastoralist never eat uncooked meat whereas Guji like to eat raw meat.

Figure 5 1 Pastoral and agro-pastoral regions of Ethiopia indicated by yellow shaded area

5

Table 5 1 Profile of Ethiopian pastoral regions

	Regional States	Pastoral area (km^2)	Population of pastoralists
1	Afar	29430	1301000
2	Benshangul Gumuz	8410	30640
3	Dire dawa	1100	108570
4	Gambela	17330	133600
5	Oromia	162070	4007950
6	SNNP*	30370	219670
7	Somali	325070	4002170
	Total	624,780	9,813,600

Source: [7]

*Southern Nations, Nationalities, and People's Region

5.1.2. Services in pastoral communities and government recognitions.

Pastoralists are geographically and socially marginalized, inhabiting large regions unsuitable for crop farming and infrastructural development. Human survival in communities in these environments would be virtually impossible without livestock that provides for basic needs. Various types of livestock, sheep, goats, cattle, camels, donkeys and horses provide nutrition, transport and clothing, and are also the basis of wealth, traditional customs and respect [3]. Pastoralist groups tend, not surprisingly, to be similar in many respects, being livestock-centred, seasonally mobile, well adapted to harsh terrain and extreme climates, tolerant of ill health, family/ clan and social network-oriented, and independent (and suspicious) of provincial or national governments[3]. They do, however, make a significant contribution to national gross domestic products (GDPs) by making marginal lands more productive. In some Sahelian countries like Burkina Faso and Sudan, 24% and 80% of agricultural Gross Domestic Product (GDP) were from pastoralism, respectively [8] and pastoralism has been shown to be from 2 to 10 times more productive than commercial ranching under the same conditions [9].

In Ethiopia the lowland pastoral production system is one of the major production systems in the country, with a major share of contribution to its economy. It was estimated that the livestock sector in Ethiopia constitutes 16% of the total Gross Domestic Product (GDP), one-third of agricultural GDP, and 8% of export earnings. Pastoralists raise the largest size

of the national livestock resource, accounting for more than 28% of the cattle, 26% of the sheep, 66% of the goats and 100% of the camels [10].

Conversely, in many countries, pastoralists lag behind settled people in education and access to public services. They are often underrepresented in governmental institutions and thus lacking political empowerment. Health and poverty initiatives at national level neglect pastoralists because of their geographic remoteness, poor communications, logistic requirements, uncertain civil status and they perceived low priority [3].

The health status of pastoral communities or populations is usually poor, and the range of infectious diseases prevalent in pastoral populations may vary with region, but tends to include similar groupings of non-zoonotic infections, such as acute respiratory and gastrointestinal (GI) infections, vaccine preventable diseases, sexually transmitted infections and some parasitic infections [11]. In addition, several zoonotic diseases appear to occur with increased frequency due to the close contacts between humans and their domesticated animals [3].

In Ethiopia over the past 10 years the significant attention has been drawn to the pastoral areas and a number of non governmental pastoralist concern organizations has been emerged and started annual pastoralist day celebration since 1998. The pastoralist day celebration was recognized by Ethiopian government and international institution, where pastoralist elders from different regions and ethnic groups, government officials and experts come together to share experiences and discuss pastoralist issues. More over the pastoralist affairs standing committee was established in 2002 at the parliament level [12]. Regardless of the effort so far made the health service of Ethiopian pastoral communities and their livestock is poor in general with varying proportion of coverage, depending on regions, ethnic groups, accessibility, geographical locations and distance from centre. During above mentioned period a number of pastoralist forums and workshops has been organized and discussed on pastoralists' issues. However, most of those forum discussions were focused on natural resources and border conflict mitigation and human and livestock health issues are often neglected.

The southeast Ethiopian pastoral area for the present study has the highest livestock population densities in the country and is the major source of Boran cattle breed and provided domestic and export markets. On the other hand human and livestock health

service delivery are constrained by number of factors of which inadequate trained professionals from pastoral communities and unwillingness of professionals to work in harsh pastoral environment. Majority of health services are delivered by community health workers and traditional healers because the present government system does not sufficiently reach pastoralists and their herds, particularly during migration seasons. In addition, in Ethiopia there is no coordinated diseases control program among animals and human health services. The Ministry of Health is responsible for human diseases control while the department of animal health under the agency for animal health and marketing is responsible for the diseases control in animals. Both Ministries are making their efforts independently and there is no officially integrated diseases control program.

5.2 One health and its concept

Calvin Schwabe coined the term "one medicine", to focus of attention on the relationship between human and veterinary health interests. His concept of "one medicine" was the general science of all human and animal health as it builds on a common pool of knowledge from anatomy, physiology, pathology, epidemiology, and aetiology in all species[13]. He showed the outcomes and potential benefits of the "one medicine" as added value to public health that could not be achieved by the disciplinary approaches alone [13,14]. A wider approach to health and well-being of societies lead to the extension of original concept of "one medicine" to "one health" through practical implementations and careful validations in different settings [15]. Recently growing recognition of the mutual interdependence of people, animals, and their ecosystems, comparable unifying concepts and institutional developments have emerged. Implications of such approach for integrated human and animal health systems are for example the simultaneous study of zoonoses in people and animals, and intersectoral health economic assessments[14]. Zinsstag et al. (2009) proposed elements of an open tool box translating the one health concept into practical method in the fields of integrated diseases surveillance, joint animal-human epidemiological studies and health services development for mutually agreed practical cooperation between human and animal health with special emphasis on developing countries. Numerous studies have shown that combining of human and animal health intervention substantially reduce the cost of zoonotic diseases control as compared to single sector specific program. Some examples of such studies were, the economics of livestock

vaccination against brucellosis in Mongolia [16], combined vaccination program through sharing of logistics and equipment between physicians and veterinarians for Chadian nomadic pastoralist and their livestock [17], a pilot study comparing cost-effectiveness of mass vaccination of dogs and post exposure treatment of human against rabies in N'Djaména [18]. In this way a combined effort could reduce cost of intervention, the time to detect emerging zoonoses and accelerate their control and prevention. Experiences from Africa and central Asia show that communication on zoonoses is often totally lacking between public-health and veterinary authorities [14].

In Ethiopia, some of known zoonotic diseases are anthrax, rabies, brucellosis, bovine tuberculosis, hydatidosis, cysticercosis and Q-fever. Pandemic influenza H1N1 and avian influenza H5N1 were also reported. Collaboration between veterinary and public health sector was initiated following the recent outbreaks of highly pathogenic avian influenza (HPAI) in East Africa. Government has created task forces between the concerned ministries of agriculture, livestock production and health for HPAI early warning and diseases surveillance. The budget proposed for combined preparedness was 70% for human health and 30% veterinary costs [19]. The extension of this approach to other zoonotic diseases control program and initiation of interdisciplinary collaborations and communications in all aspects of health care for humans, animals and the environment would be essential for Ethiopia like other African countries.

The simultaneous study of mycobacteria in humans and livestock, brucellosis and Q-fever in animals in the present study demonstrates an added value of a "One Health" approach of closer cooperation of human and animal health sectors.

5.3.1. Classification of mycobacteria

The genus *Mycobacterium* is ever expanding and it has more than 100 well-characterized species [20, 21].This review is to give a brief overview of members of *Mycobacterium tuberculosis*-complex, *Mycobaterium avium*-complex and nontuberculous mycobacteria.

5.3.2 The Mycobacterium tuberculosis-complex (MTC)

The mycobacteria grouped in the MTC are characterized by 99.9% similarity at the nucleotide level and identical 16S rRNA sequences but differ widely in terms of their host tropisms, phenotypes, and pathogenicity [22,23] suggesting that they all derived from a

common ancestor [24]. MTC species, namely, *M. tuberculosis*, *M. africanum, M. microti*, *M. canetti,* and *M. bovis*, can be categorized according to a restricted number of laboratory phenotypes and genetic markers but, importantly, differ in physiological characteristics, virulence and host range [25,26]. Though it has been conventionally established that *M. tuberculosis* and *M. africanum* are isolated from humans, *M. microti* from voles, and *M. bovis* predominantly from cattle, reports of MTC organisms in a variety of other domesticated and wildlife hosts pose a challenge to this classification scheme [26]. *M. microti* causes tuberculosis (TB) mainly in small rodent-like voles, but until now its importance for TB in humans has remained unclear [25]. The *M. canetti* can cause TB in humans, but so far only a few *M. canetti* strains have been isolated and its epidemiological contribution to TB in humans is uncertain[27].

The two subgroups of *M. africanum* have been described as subtype I and II. Numerical analyses of their biochemical characteristics revealed that *M. africanum* subtype I is more closely related to *M. bovis*, whereas subtype II more closely resembles *M. tuberculosis* [28,29]. Thus *M. africanum* is an intermediate species between *M. tuberculosis* and *M. bovis*. The host range of *M. bovis* is considered to be the broadest of the complex, causing disease across a variety of animals. Other member of MTC is the *M. caprae* that isolated from goats[30, 31]. The *M. pinnipedii* is also species in the MTC, based on host preference, phenotypic and genotypic characteristics. *M. pinnipedii* has been isolated mainly from sea lions and seals [32]. Recentlly, Alexander et al [33] reported *M. mungi* as new member of MTC causing disease out break in banded mongooses in Botswana (Figure 5.2).

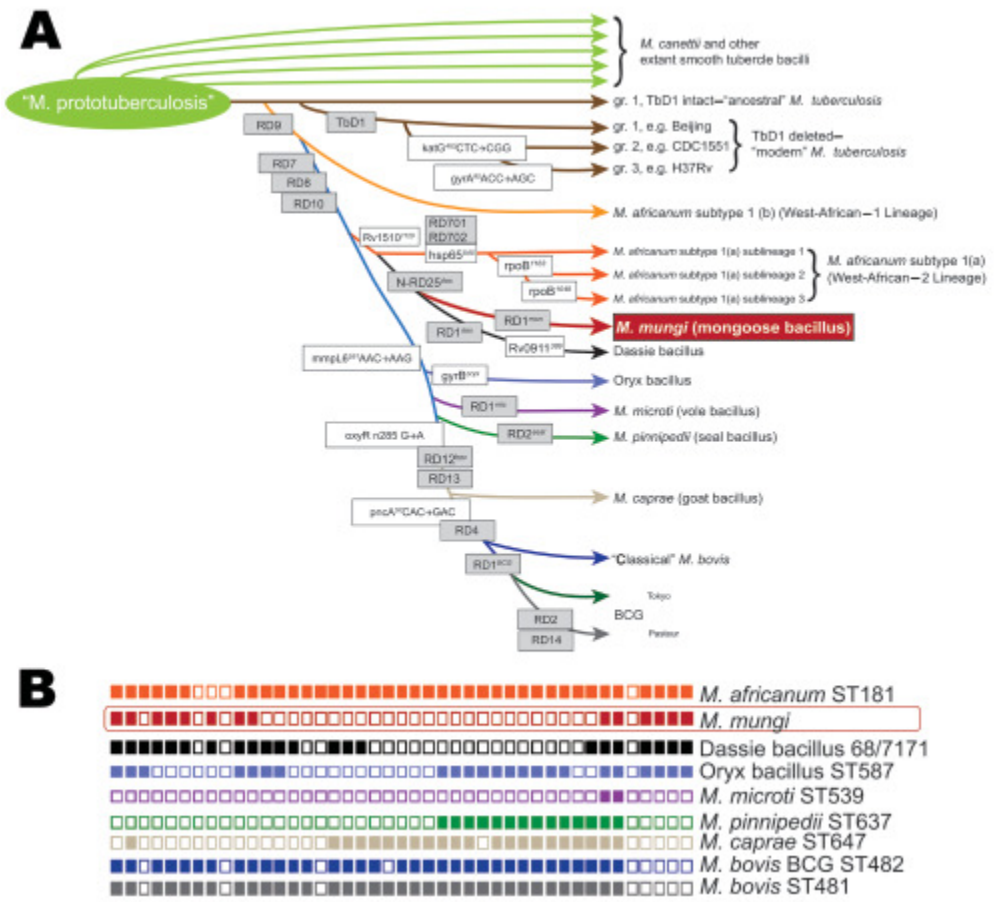

Figure 5 2 Schematic of the phylogenetic relationships among Mycobacterium tuberculosis complex & newly discovered *M. mungi*.

A) Schematic of the phylogenetic relationships among *Mycobacterium tuberculosis* complex species, including newly discovered *M. mungi,* based on the presence or absence of regions of difference (gray boxes) as well as specific single-nucleotide polymorphisms (white boxes), modified from [34]. B) Spoligotype of *M. mungi* compared with representative spoligotypes from other *M. tuberculosis* complex species [33]

More recently clinically and epidemiologically important *M. tuberculosis* strains has been classified based on molecular characterization by using single nucleotide polymorphism(SNP) and large sequence polymorphism (LSP) [35,36]. Based on LSP Gagneux et al (2006) classified *M. tuberculosis* into six lineages with its geographical association, namely Indo-Oceanic lineage (1) in East Africa, Southeast Asia, and south India; East Asian lineage (2) in East Asia, Russia and South Africa; East African-Indian lineage (3) in East Africa, north India and Pakistan; Euro-American lineage (4) in Americas, Europe, north Africa and Middle east; West African lineage I (5) in Ghana,

Benin, Nigeria and Cameroon and West African lineage II (6) in Senegal, Guinea-Bissau and The Gambia. Gagneux et al classification of six lineages is under revision due to investigation of novel Ethiopian lineage proposed as lineage 7th lineage (unpublished data).

5.3.3 The Mycobacterium avium-complex (MAC)

The *M. avium* complex (MAC) consists of genetically similar, slowly growing bacteria that are divided into the two opportunistic pathogenic species *M. avium* and *M. intracellulare* [37], and non-well characterised, closely related to non-pathogenic organisms. *M. avium* is further divided into 4 subspecies: *M. avium* subsp. *avium*, *M. avium* subsp *paratuberculosis*, *M. avium* subsp. *silvaticum* and *M. avium* subsp. *Hominissuis* [38-40]. Although the real *M. avium* species have never been isolated successfully from the environment, *M. intracellulare* and the closely related mycobacterial species can be isolated from multiple environmental sources, including drinking water, soil, plants [38, 39].

Although human exposure to MAC is ubiquitous, most individuals rarely develop infections. Immuno-compromised individuals, such as those with HIV infection or individuals who have had organ transplants, are at the greatest risk for MAC infection. Before the emergence of AIDS, most MAC infections were pulmonary in nature and typically affected patients with preexisting lung diseases, such as emphysema or cystic fibrosis [20, 38]. MAC is also the most frequent cause of pediatric cervical lymphadenitis [41].

Although *M. avium* subsp. *avium* and *M. intracellulare* are the main causative agents of human MAC diseases [38], *M. avium* subsp. *Paratuberculosis*, the etiological agent of paratuberculosis or Johnes disease in ruminants[39], has been suggested to be an emerging human pathogen responsible for Crohn's disease [42]. In addition to the well-known MAC infections, members of MAC are implicated as causes of human granulomatous diseases, such as sarcoidosis [38].

5.3.4 The nontuberculous mycobacteria (NTM)

The genus *Mycobacterium* comprises both the strictly pathogenic species that are transmitted by human or animal reservoirs and the so-called nontuberculous mycobacteria (NTM) [21, 43]. Nontuberculous mycobacteria (NTM) are ubiquitous organisms with nearly 100 different species found in soil and water [44]. These other mycobacteria are also referred to as atypical mycobacteria or mycobacteria other than the *Mycobacterium tuberculosis*

complex (MOTT) [20]. Environmental nontuberculous mycobacteria species that are not members of the *M. tuberculosis* complex are ordinary inhabitants of a wide variety of environmental reservoirs and their role in human and animal diseases has been fully recognized [39]. The members of NTM are usually saprophytes but can be opportunistic and at times deadly pathogens especially to immunosuppressed persons [20, 43]. Several NTM constitute a risk not only to immunosuppressed persons but also to otherwise healthy persons [43]. These organisms can produce localized disease in the lungs, lymph glands, skin, wounds or bone. Occasionally they may produce disseminated disease [20].

About one third of NTM are associated with disease in humans. The species causing human disease are: *Mycobacterium avium, M. intracellulare, M. kansasii, M. avium subsp. paratuberculosis, M. scrofulaceum, M. simiae, M. habana, M. interjectum, M. xenopi, M. heckeshornense, M. szulgai, M. fortuitum, M. immunogenum, M. chelonae, M. marinum, M. genavense, M. haemophilum, M. celatum, M. conspicuum, M. malmoense, M. ulcerans, M. smegmatis, M. wolinskyi, M. goodii, M. thermoresistible, M. neoaurum, M. vaccae, M. palustre, M. elephantis, M. bohemicam and M. septicum* [20]. Isolation of these mycobacteria from representative specimens and their rapid identification is very important as the treatment strategy for tuberculosis and other mycobacterioses is different [20, 45]. In some of Western African countries, *M. ulcerans*, causing Buruli ulcer, is the third most common mycobacterial infection. Moreover, *M. leprae* is one of important mycobacteria in Ethiopia, causing leprosy in human.

5.4.1 Tuberculosis

Globally, tuberculosis (TB) causes 1.4 millions deaths per year and 9.4 millions people with disease [46]. Most of global TB cases occurred in Asia (55%) and Africa (30%). Smaller proportions of cases occurred in the Eastern Mediterranean Region (7%), the European Region (4%) and the Region of the Americas (3%). Twenty two high burden countries account for 81% of all worldwide TB cases estimate [47]. WHO set global targets for reducing and control of the TB burden for 2015 and 2050. Currently, most attention is given to the targets set for 2015. This target is within the context of the millennium development goal to halt and reverse the incidence of TB by 2015. The additional targets set by the Stop TB partnership are to halve TB prevalence and death rates by 2015, compared with their levels in 1990. The Stop TB Strategy is the approach recommended by

WHO to reduce the burden of TB in line with global targets set for 2015. The six major components of this strategy are: (i) pursue High-quality DOTS expansion and enhancement; (ii) address TB/HIV, multi-drug resistant TB, and the needs of poor and vulnerable populations; (iii) contribute to health-system strengthening based on primary health care; (iv) engage all care providers; (v) empower people with TB, and communities through partnership; and (vi) enable and promote research[47].

About 50 year ago tuberculosis has been identified as one of the major public health problems in Ethiopia and effort to control tuberculosis began in the early 1960s with the establishment of TB centers and sanatoria in three major urban areas in the country [48]. In Ethiopia, TB is the leading cause of morbidity, the third cause of hospital admission after deliveries and malaria, and the second cause of death, after malaria. It is an obstacle to socio-economic development; 75% of people affected by TB are within the economically productive age group of 15-54 years [49].

Currently, Ethiopia ranks seventh among the world's 22 countries with high tuberculosis burden and had an estimated incidence of all forms and smear positive TB were 379 and 168 cases per 100,000 people per year, respectively. The prevalence and mortality of TB of all forms is estimated to be 643 and 84 per 100,000 populations, respectively [46]. Extra-pulmonary tuberculosis (EPTB) account for 34% of all TB forms [48] and the reason for high EPTB prevalence are unknown.

To combat TB, Ethiopia was introduced DOTS in 1992 and reached 90% geographical coverage (it means that 90% of Woreda/Districts are covered by DOTS service); the health facilities coverage is 72.3%, hence, given the limited infrastructure in the country, only 60% of the population has access to DOTS services. In order to reach national targets, TB control program of Ethiopia is aligned with the globally recommended Stop TB Strategy and the basic strategies are: early case detection, adequate chemotherapy, provision of comprehensive & standard patient care, enhanced case management, accurate Monitoring and Evaluation (M & E) of program performance and community participation [49]. To improve its health service delivery, Ethiopia has re-structured its system in three-tier, which characterized by a first level of a Woreda/District health system comprising a primary hospital (with population coverage of 100,000 people), health centers (1/25,000 population) and their satellite health posts (1/5,000 population) connected to each other by

a referral system. A primary hospital, health centers and health posts form a primary health care unit with each health centre having five satellite health posts. The second level in the tier is a General Hospital with population coverage of one million people; and the third a Specialized Hospital that covers a population of five million[48]. In 2004, the government of Ethiopia launched the Health Extension Program to improve equitable access to preventive essential health services. These services are being offered by Health Extension Workers based in Health Posts located in each kebele (the lowest government administrative unit). Women selected by the community were trained for 1 year covering the 4 components of the health program: Hygiene and Environmental Health, Family Health Services, Disease Prevention and Control and Health Education and Communication. The role of health extension workers to fight against TB was limited to health education, referral of suspected cases and defaulter tracing. Recently, Federal Ministry of Health decided to increase the responsibilities of the health extension workers in the prevention and control of tuberculosis. The main reasons for this decision were the persistent low case detection rates of TB cases and the introduction of Rifampicine-Isoniazide to replace Ethambutol-Isoniazide as continuation phase [50]. More recently, government introduced TB control program at community level (community DOTS) in 3 regions as pilot program and expected to scale up to other regions. The major implementers of community DOTS are Health Extension Workers and community members and community organizations [49].

Ambitious government strategic plans to control TB is far to achieve its objectives in a remote rural areas in general and pastoral communities in particular. Both intervention and TB research activities are limited in pastoral settings. Information on TB situation in Ethiopian pastoralist area is limited both in humans and livestock. More importantly incidence and prevalence rates of human TB due to *M .bovis* is unknown.

The unusually high extra-pulmonary and cervical TB lymphadenitis cases in Ethiopia, prevalent BTB in cross-breed dairy farm in central Ethiopia and living style of close contact between livestock and human in rural communities, initiated a Wellcome Trust program to study epidemiology of BTB in Ethiopia. Initaily the program was focused on the isolation and characterization of TB isolates from fine needle asprite (FNA) and TB suspected lesions from various abattoirs in the north and central Ethiopia. At a later stage isolates from pulmonary TB patients were included to Wellcome Trust BTB study

program. The present study is the pastoral component of the Wellcome Trust BTB study program in Ethiopia.

5.4.2 Bovine Tuberculosis

Bovine tuberculosis is caused by *M. bovis* in cattle and other mammals including man and is a member of the *Mycobacterium tuberculosis* complex [51]. A phylogeny of the *Mycobacterium tuberculosis* complex has recently shown that the animal-adapted strains are found in a single lineage marked by the deletion of chromosomal region (RD9) [24, 52]. The classical *M. bovis* showed the greatest number of RD deletions and seem to have undergone the greatest loss of DNA relative to other members of the *M. tuberculosis* complex. These lacked regions RD4, RD5, RD6, RD7, RD8, RD9, RD10, RD12, and RD13 [24] and it is thought that *M. bovis*, is the most recent member of this lineage[51] (figure 5.3).

Smith and his colleagues applied the ecotype concept to strains of the RD9 deleted lineage. Strains of this lineage occupy a series of clades each with a different host preference representing different niches. There are molecular differences between the clades that were present in the most recent common ancestor of the clade and, therefore, present in all descendants. In this approach, the successive and unidirectional loss of DNA in representative strains reveals the order in which members of the complex descended from their ancient common ancestor [52].

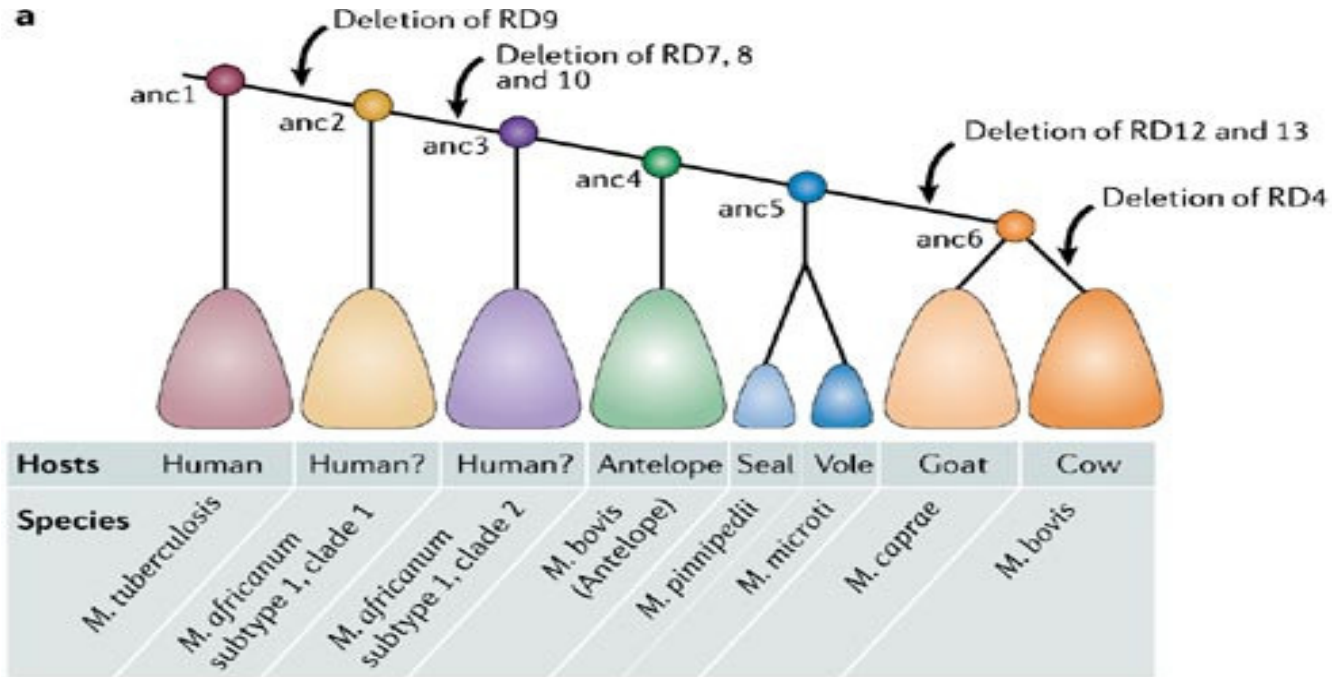

Figure 5 3 The distribution of phylogenetically informative deletions that form the backbone of phylogeny and lineage leading to M. bovis.

Species and subspecies designations are shown, as well as the most likely host for each ecotype.The members of the *Mycobacterium tuberculosis* complex (excluding *Mycobacterium canettii*) are shown as a series of clades, containing host-adapted ecotypes[52], distinguished by phylogenetically informative mutations that are fixed in all descendant clades. Coloured circles, marked anc1 to anc6, represent single-cell ancestors and imply severe bottlenecks in the diversity of this lineage; the representation of each clade does not necessarily reflect the diversity in each clade [51, 52].

5.4.2.1 Clonal complexes of M. bovis

Recent molecular studies based on deletion analysis of specific chromosomal regions and absence of specific spacers in standard spoligotype patterns revealed that certain *M. bovis* strains occurr in high frequency in cattle population of specific regions. They are characterized as clonal complex of *M. bovis* and epidemiologically dominant and geographically localized in the occurring regions. Some of such clonal complexes of *M. bovis* that have been identified are African 1 (Af1) clonal complex [53], African 2 (Af2) clonal complex [54] and European 1 (Eu1) clonal complex [55].

Af1 clonal complex present at high frequency in cattle population of west-central African countries and localized in Mali, Cameroon, Nigeria, and Chad and yet not identified from north, south and eastern African countries. They are closely related and defined by a specific chromosomal deletion (RDAf1) and the absence of spacer 30 in the spoligotype

patterns [53]. Af2 clonal complex occurs in cattle of eastern African countries, such as Uganda, Burundi, Tanzania, and Ethiopia. Af2 strains are defined by a specific chromosomal deletion (RDAf2) and by the absence of spacers 3 to 7 in their spoligotype patterns [54]. About 99% of BTB in the Republic of Ireland and the UK is caused by EU 1 clonal complex. This strain is present at low percent in mainland European countries but found at high frequency in former trading partners of the UK (USA, South Africa, New Zealand, Australia and Canada). The Americas, with the exception of Brazil, are dominated by the Eu1 clonal complex which was at high frequency in Argentina, Chile, Ecuador and Mexico as well as North America. Eu1 is rare or absent in the African countries except South Africa. EU 1 strains are marked by the deletion of chromosomal region RDEu1 and absence of spacer 11 in spoligotype pattern [55]. As compared to Af1 and Af2 strains EU1 is widely distributed and epidemiologically important clonal complex of *M. bovis*.

The spoligotype patterns of *M. bovis* isolates from cattle and TB patients in the present study is characterized by absence of spacers 3 to 7 as mentioned in Berg et al (2011).

5.4.2.2 Epidemiology of bovine tuberculosis

Although bovine tuberculosis was once found worldwide, control programs have eliminated or nearly eliminated this disease from domesticated animals in many countries [56]. Nations currently classified as Bovine tuberculosis-free include Australia, Iceland, Denmark, Sweden, Norway, Finland, Austria, Switzerland, Luxembourg, Latvia, Slovakia, Lithuania, Estonia, the Czech Republic, Canada, Singapore, Jamaica, Barbados and Israel. Eradication programs are in progress in other European countries, Japan, New Zealand, the United States, Mexico, and some countries of Central and South America [57]. Even though bovine tuberculosis has been eradicated from the majority of U.S. states, a few infected herds continue to be reported, and a few states may periodically lose their disease-free status. In particular, a focus of infection in wild white-tailed deer has complicated eradication efforts in Michigan. Similar problems exist with infected badgers in the U.K. and Ireland, and infected brush-tailed opossums in New Zealand[39, 58-60]. Bovine tuberculosis is still widespread in Africa, parts of Asia and some Middle Eastern countries [56, 61].

5.4.2.3 Animal reservoirs

Cattle are the primary hosts for *M. bovis*, but other domesticated and wild mammals can also be infected. Known maintenance hosts include brush–tailed opossums (and possibly ferrets) in New Zealand, badgers in the United Kingdom and Ireland, bison and elk in Canada[57,59], and kudu and African buffalo in southern Africa[62,63]. White-tailed deer in the United States (Michigan)[60] have been classified as maintenance hosts; however, some authors now believe they may be spillover hosts. Species reported to be spillover hosts include sheep, goats, horses, pigs, dogs, cats, ferrets, camels, llamas, many species of wild ruminants including deer and elk; elephants, rhinoceroses, foxes, coyotes, mink, primates, opossums, otters, seals, sea lions, hares, raccoons, bears, warthogs, large cats (including lions, tigers, leopards, cheetahs and lynx) and several species of rodents[57].

BTB is endemic in Ethiopia, but the role of wildlife in maintaining BTB is unknown. Livestock and wildlife share pasture, water sources, mineral licking areas and contact at grazing area and premises during night. Tschopp et al [64] have studied BTB status in Ethiopian wildlife populations in five regions using rapid serology test (RT) based on lateral flow technology, and culturing of lymph node specimens. Twenty three percent of tested sera by RT and 32.5% cultured specimens were acid-fast bacilli positive. None of culture positive isolates was identified as MTC [64].

5.4.2.4 Clinical and pathological aspect

There are various ways in which cattle can become infected with *M. bovis*; these can be affected by animal age and behavior, environment and climate, and prevailing farming practices [65] . Lesion distribution and pathology show predominant involvement of the upper and lower respiratory tract and associated lymph nodes [66, 67]. Characteristic tuberculous lesions occur most frequently in lungs and retropharyngeal, bronchial and mediastinal lymph nodes. Lesions can also be found in the mesenteric lymph nodes, liver, spleen, serous membranes, pleura and other organs [66]. The characteristic lesion caused by *M. bovis* in cattle is described as having a centre of caseous necrosis, usually with some calcification, with a boundary of epithelioid cells, some of which form multinucleated giant cells and few to numerous lymphocytes and neutrophils. Primary lesions in cattle, unlike man, are rarely contained by the immune response, and dissemination from a lesion may occur by natural

ducts such as bronchi, by lymphatic spread or by haematogenous spread when massive miliary TB occurs [61].

Human TB caused by *M. bovis* is clinically indistinguishable from TB caused by *M. tuberculosis* clinically, radiologically and pathologically[39,68]. In countries where bovine TB is uncontrolled, most human cases occur in young persons and result from drinking or handling contaminated milk; cervical lymphadenopathy, intestinal lesions, chronic skin TB (lupus vulgaris), and other nonpulmonary forms are particularly common. Such cases may, however, also be caused by *M. tuberculosis* [56].

5.4.2.5 BTB Diagnosis

TB can be diagnosed clinically, but usually only in the later stages of the disease. The tuberculin skin test is universally recognized and is generally used for preliminary diagnosis in bovine TB control programs. However, in countries with low disease prevalence or disease free status, meat inspection is used for diagnosis and surveillance. Other tests, such as an antibody enzyme-linked immunoassay (ELISA) and the gamma-interferon assay, have been used as supplementary tests in eradication and control [61]. Bovine tuberculosis infection in cattle is usually diagnosed in the live animal on the basis of delayed hypersensitivity reactions (tuberculin skin testing). In cattle, infection is often sub-clinical; when present, clinical signs are not specifically distinctive to other disease-caused conditions and might include weakness, anorexia, emaciation, dyspnoea, enlargement of lymph nodes, and cough, particularly with advanced TB [69]. Post-mortem, infection is diagnosed by necropsy and histopathological and bacteriological techniques. Rapid nucleic acid methodologies such as the polymerase chain reaction (PCR) may also be used. These are demanding techniques and only validated procedures should be used. Classical mycobacterial culture remains the routine method for confirmation of infection [69]. Delayed hypersensitivity test is the standard method for detection of BTB in live animals. It involves measuring the skin thickness, injecting purified protein derivatives, bovine tuberculin (PPD-B) intradermally into the measured area and measuring any subsequent swelling at the site of injection three days later. The comparative intradermal tuberculin test with bovine and avian tuberculin is used mainly to differentiate between animals infected with *M. bovis* and those sensitized to tuberculin due to exposure to other mycobacteria or related genera. The recommended dose of bovine (PPD-B) in cattle is at least 2000

International Units (IU) and in the comparative tuberculin test, the doses should be no lower than 2000 IU each. The reactions are interpreted on the basis of appropriate schemes [69]. OIE recommend the cut-off point for positivity of the comparative intradermal tuberculin calculated as the difference between skin thicknesses after 72 hours of bovine tuberculin (PPD-B) and avian tuberculin (PPD-A) injections (*B _ A*), is greater than 4 mm. However, currently the use of a cut-off greater than 2 mm has been suggested as an appropriate depending on local situation in Africa[70-72] since they have shown that the sensitivity increases at virtually the same specificity at this cut-off. Other available tests in live animal are blood-based laboratory tests. There are gamma interferon assay, lymphocyte proliferation assay, and enzyme-linked immunosorbent assay [69].

In the gamma interferon assay, the release of a lymphokine gamma interferon (IFN-γ) is measured in a whole-blood culture system. The assay is based on the release of IFN-γ from sensitized lymphocytes during a 16–24-hour incubation period with specific antigen (PPD-tuberculin). The test makes use of the comparison of IFN-γ production following stimulation with avian and bovine PPD. It is recommended that the blood samples must be transported to the laboratory and the assay set up as soon as practical, but latest within 8–12 hours of collection [69]. The gamma-interferon assay is considered to have a high sensitivity compared with the skin test and acceptable level of specificity. In animals that are difficult or dangerous to handle, such as excitable cattle or other bovidae, it has the advantage over the skin test in that the animals need only be captured once for blood sampling [73].

Lymphocyte proliferation assay is an invitro assay that compares the reactivity of peripheral blood lymphocytes to bovine tuberculin PPD-B and avian tuberculin PPD-A. The assay can be performed on whole blood or purified lymphocytes from peripheral blood samples. These tests endeavour to increase the specificity of the assay by removing the response of lymphocytes to ‘nonspecific’ or cross-reactive antigens associated with non-pathogenic species of mycobacteria to which the animal may have been exposed. Results are usually analyzed as the value obtained in response to PPD-B minus the value obtained in response to PPD-A. The lymphocyte proliferation assay test has been reported to have a high sensitivity and specificity in diagnosis of *M. bovis* infection in deer; however, the test is relatively expensive and has not yet been subject to inter-laboratory comparisons [69].

Enzyme-linked immunosorbent assay (ELISA) appears to be the most suitable of the antibody detection tests and can be a complementary test, rather than an alternative, to tests based on cellular immunity. It may be helpful in anergic cattle and deer. An advantage of the ELISA is its simplicity, but both specificity and sensitivity are limited in cattle, mostly due to the late and irregular development of the humoral immune response in cattle during the course of the disease [74,75]. Fluorescence polarization assay (PFA) also constitutes an alternative technique for antibody detection with a shown potential for diagnostic purposes. However, Ngandolo et al (2009) demonstrate that a test is unsuitable for the detection of animals with gross visible lesions.

5.4.2.6 M. bovis in human

Following the introduction of milk pasteurization and large scale test and slaughter programs in cattle, the occurrence of BTB became rare in humans and cattle in industrialized countries [56, 61]. However, it remains an important disease in many countries of the world where BTB is endemic, causing significant economic losses [76]. BTB in animals has been reported from 33 of 43 African countries [61]. Human bovine tuberculosis cases have so far been described in some Sahelian countries like Ghana, Niger, Uganda and Tanzania[77-79] and in immigrants from Chad to France[80]. The representative proportion of BTB in human tuberculosis is estimated at less than 5% worldwide [56, 81].

5.4.2.7 BTB in Ethiopia

In Ethiopia, BTB is endemic in cattle. Prevalence varies depending on the geographical areas, the breeds and the husbandry practices. In Central Ethiopia, BTB surveys showed an abattoir prevalence of 3.5% to 5.2% and prevalence in dairy farms with cross-breeds varying between 3.5% and 50% [82-85]. Prevalence in traditionally kept zebu cattle varies between 0.9-4% based on cut-off value used for interpretation [86]. Mamo et al [87] reported 5% prevalence of gross tuberculous lesion in camels slaughtered at Dire Dawa abattoir in eastern Ethiopia. Hik and Agga [88] reported 4.2% abattoir prevalence of bovine TB in Mojo export abattoir base on gross lesions.

Tschopp et al [89] studied prevalence of BTB at human-livestock-wildlife interface in South Omo pastoralists in Hamer Woreda of South-West Ethiopia and reported individual BTB prevalence in cattle as 0.8% with the >4 mm cut-off and 3.4% with the >2 mm cut-off.

5.4.2.8 BTB Control options

In industrialized countries, control and eradication of bovine TB has been successfully carried out by regular testing and slaughter of infected animals under mandatory national bovine TB program [56, 61]. Such programs have been successful in many European Union member states and in seven central European countries between 1953 and 1980. In developing countries, however, bovine TB remains a major animal health problem, mainly because these countries cannot shoulder the financial burden required to implement a control program and compensate for slaughtered animals [61]. Limited access to education, poor information networks and lack of disease surveillance are other factors that limit the implementation of such program [90]. Vaccination of animals against TB would be a viable strategy in two disease control situations: in domesticated animals in developing countries and in wildlife and feral reservoirs of disease in industrialized countries where test-and-slaughter programs have failed to achieve elimination of the disease [56].

Like other African countries control strategies for BTB in Ethiopia is also limited to the inspection and condemnation of infected carcasses at slaughterhouses. There is no policy in place for test and slaughter as well as vaccination program. The Ethiopian Federal Ministry of Agriculture in collaboration with the current Wellcome Trust BTB project in Ethiopia has implemented test and removal method in one government owned cross-breed dairy farm in Holota (central Ethiopia). This experience can be taken as model for the future control strategies implementation in dairy farms in similar situation. Further more, the Wellcome Trust BTB research project in Ethiopia is in the final stage of its multi-disciplinary TB research work and expected to generate cost of BTB intervention and evidence based appropriate control options.

5.5 Brucellosis and Q-fever

Brucellosis is a disease of animals, especially domesticated livestock (cattle, goats, sheep, camels and pigs) and other animals. It is caused by bacteria of the genus *Brucella* spp.. In animals it is primarily a reproductive disease, characterised by late abortion, retained foetal membranes, orchitis and impaired fertility [91]. *B. melitensis* is considered to have the highest zoonotic potential, followed by *B. abortus,* and *B. suis*. Brucellosis remains one of the most common zoonotic diseases worldwide with more than 500,000 human cases reported annually specially in developing countries[92-94].

The economic and public health impact of brucellosis remains of particular concern in developing countries [16]. The disease poses a barrier to trade of animals and animal products, causes a public health hazard, and is an impediment to free animal movement [91]. In general, the incidence is higher in livestock raised under pastoral production systems [95]. Brucellosis is endemic in humans and livestock in the Mediterranean region, Africa, the Near East, and Central America [91]. It is re-emerging as a major epidemic in countries of the former Soviet Union [16]. Previously serological study of brucellosis has been carried out in farm animals in Ethiopia. The results have shown that its presence in livestock varies between different parts of the country [96-99]. Only few serological studies of brucellosis have demonstrated the occurrence of the disease among Borana and Hamer pastoralists highlighting the public health significance [100].

Q-fever is a zoonotic disease caused by *Coxiella burnetii*. Farm animals (cattle, sheep, camels and goats) and pets are the main reservoirs of infection and transmit to humans [101]. Q-fever is known to be transmitted mainly by ticks among domestic animals. The mechanisms by which it is transmitted from livestock to humans are very similar to those of brucellosis. They have been shown to include consumption of unpasteurized dairy products, direct contact with diseased animals and their carcasses, or with secretions aborted materials via respiratory and conjunctival route [102]. Infection in humans is often asymptomatic, but it can manifest as an acute disease (usually a self-limited flu-like illness, pneumonia or hepatitis) or as a chronic form (mainly endocarditis, but also hepatitis and chronic-fatigue syndrome). Q-fever was rarely reported and is frequently misdiagnosed by physicians [101]. It is endemic both in livestock and humans in North and Sub-Saharan Africa [102-105]. In Ethiopia, the existence of antibody against *C. burnetii* was reported in goats and sheep slaughtered at Addis Ababa abattoir and its environs [106]. A seroprevalence of 6.5% was also reported in Addis Ababa abattoir workers [107] showing its public health significance. To our knowledge, there was no study of Q-fever in Ethiopian livestock under field condition in general and in pastoral areas in particular, where the people have more close contact with animals favoring zoonotic infections.

At present, the Ethiopian government's efforts focus on pastoral development to achieve food security and earn foreign currency. Pastoralists and institutions promoting the pastoral development activities require current and reliable scientific data on such diseases as

brucellosis and Q-fever. However, previous studies on both diseases have been limited in coverage and scope. The objectives of the present study were to assess the seroprevalences of brucellosis and Q-fever in pastoral livestock in southeast Ethiopia.

6. Research rationale and institutional collaborations

In industrialized countries a number of epidemiological studies on zoonotic diseases both in animals and humans have been conducted, whereas data from similar works from Africa are rare, where incidence rates of those diseases like TB are high and a close contact between cattle and people exists. However, this data is needed to provide information for the planning of bovine tuberculosis and other zoonoses control strategies both in animals and humans. In Ethiopia, the results of this epidemiological study could help in providing new innovative control strategies in general and in pastoral areas in particular. It also contributes to the estimation of the proportion of human TB infection due *M. bovis*, prevalence of mycobacteria in humans and animals, and status of brucellosis and Q-fever prevalence in the study areas.

To conduct this epidemiological study of mycobacterial infection in animals and humans and status of other zoonotic diseases in pastoral areas of southeast Ethiopia, a partnership was established between the Swiss Tropical and Public Health Institute, National Centre of Competence in Research North-South (NCCR), the Armauer Hansen Research Institute (AHRI), the Wellcome Trust funded bovine TB program in Ethiopia and the Jimma University.

The Wellcome Trust Fund BTB program in Ethiopia has different work packages, namely work package one: cross-sectional survey of *M. bovis* in Ethiopian cattle [83,108], work package two: cross-sectional survey of *M. bovis* in human lymph node[109], work package three: prevalence of BTB in Selale region/central highland of Ethiopian[108], work packages four: analysis of influence of cattle genetic background on susceptibility to BTB, work package five: experimental infection of cattle with *M. bovis*, work package six: estimation of the costs to society of BTB, and cost-benefit model of intervention, work package seven: evaluation of the impact of cattle vaccination on development of BTB[110]. These different work packages were mostly carried out in central and northern parts of Ethiopia and not sufficiently covered marginalized nomadic pastoralist areas. Therefore, present study is intended to contribute for the pastoral component of work package one and two

7. Goal and objectives

7.1 Goal

Study epidemiology of tuberculosis and other mycobacteria infections with special emphasis on bovine tuberculosis in animals and humans in the pastoral areas of southeast Ethiopia. This work is pastoral component of Wellcome Trust project on BTB in Ethiopia and contributes to the overall project work.

7.2 Objectives

-To determine the epidemiology of *M. bovis* and other mycobacteria in animals and humans in the pastoral areas of southeast Ethiopia.

- To assess the prevalence of tuberculin reactors in the pastoral livestock in south Ethiopia and to evaluate the determinants of tuberculin reacting animals.

- To assess the status of other zoonoses (brucellosis and Q-fever) among pastoral livestock.

8. Study sites, sampling and sample flows

8.1. Study sites

The field work was carried out in two pastoral areas of Guji zone (Negelle) of Oromia region and Liben zone (Filtu) of Somali region (Figure 8.1) in the southeast Ethiopia. The two study sites, Negelle and Filtu are located at 595km and 730km south-east of Addis Ababa, respectively. Guji zone comprises three agro-ecological zones, namely highlands, midlands and lowlands. The lowlands in the study area are inhabited by pastoral and agro-pastoral communities whose livelihood is based on livestock production. The Liben (Filtu) zone of Somali region is lowland and is characterized by arid agro-ecological zone and inhabited by pastoral communities. The major livestock kept by pastoralist in Guji zone are cattle, goats and camels whereas pastoralist in Liben zone of Somali region kept camels, goats and cattle in order of their livelihood importance, respectively.

A cross-sectional study of BTB in cattle, camels and goats were carried out in Hayadimtu, Bifatu, Melka-Libe and Bakaka Pastoralist association (PA) of Filtu Woreda in Liben zone of Somali region. In Guji zone of Oromia region study was conducted only in cattle and 4

PAs (Dhuko, Sirba, Arda-Bururi and Siminto) in Liben and Goro-Dola Woredas were included.

All pastoral herd owners of tuberculin-tested livestock were interviewed using pre-tested structured questionnaires. The questionnaire was pre-tested with non-study pastoral households' participants in the study area. Responses of herd owners were filled into the questionnaire form with the following parts: i) personal information ii) type of livestock owned, herd size and major cattle breeds owned iii) management practices i.e. type of housing, frequency of new animals purchased per year, dynamics of herd movement per year, common places for herd contact and frequency of contact, and other practices. In addition, a focus group discussion (FGD) was conducted at each study PAs using a semi-structured interview catalogue. The language used for questionnaire interviews and FGD was locally spoken *Afan* Oromo language in Guji zone and Somali language in Liben zone.

During tuberculin testing, sera samples were collected from all tuberculin-tested animals for brucellosis and Q-fever testing. Sera samples were tested by Rose Bengal Plate Test (RBT) and enzyme-linked immunosorbent assay (ELISA) for brucellosis, and ELISA test for Q-fever.

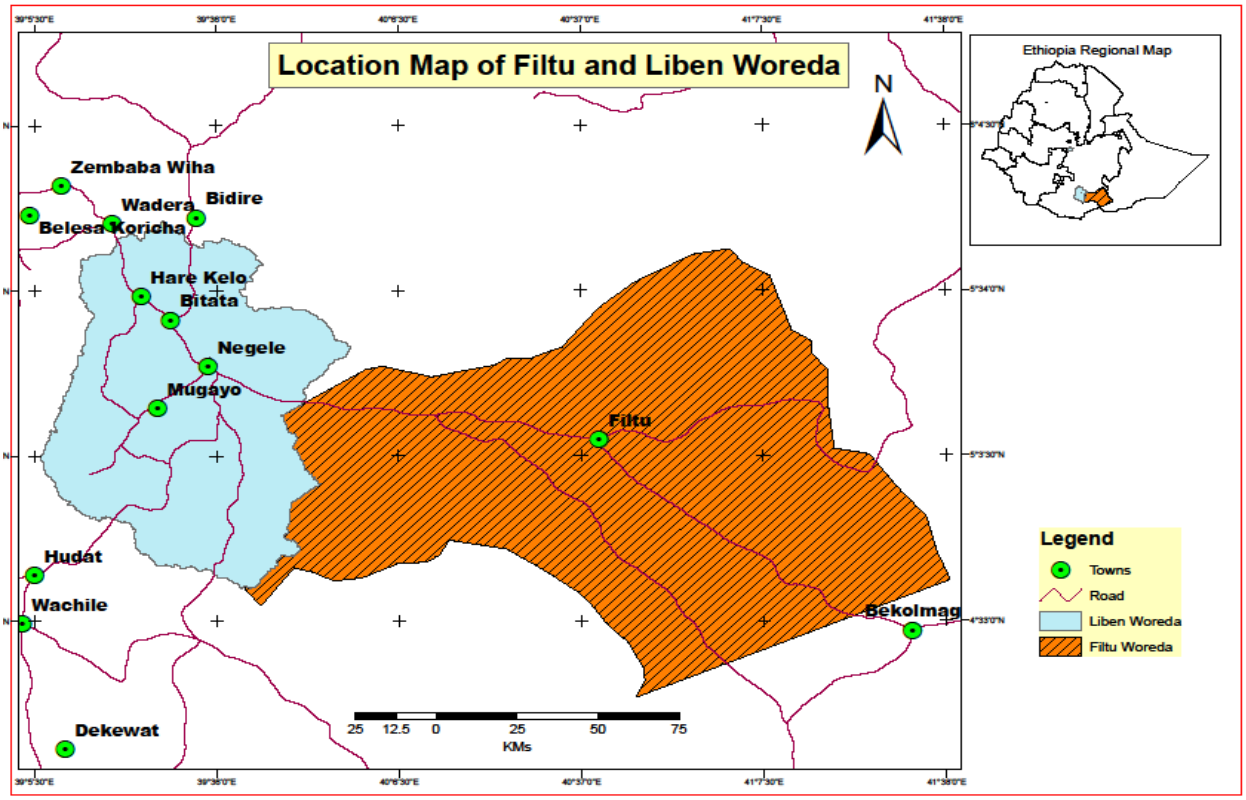

Figure 8 1 Location map of study area

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8.2 Sampling and sample flows

TB suspected lesions from livestock (cattle, camels and goats) slaughtered in study area, sputum from pulmonary TB patients and fine needle aspirates (FNA) samples from TB lymphadenitis were collected and processed at Armauer Hansen Research institute. Culture positive isolates were characterized by molecular typing techniques.

The following chart (Figure 8.2) summarizes sampling and sample flows.

Field works
- PPD skin testing

Blood serum has been collected from all PPD tested animals for;
- Brucellosis testing and
- Q-fever testing

Abattoir specimens
- Cattle
- Camels
- Goats

Human specimens
- Sputum
- FNA

AHRI laboratory
Growth cultures, ZN staining, heat-killed cells

Brucellosis
RBT –all serum

ELISA test- all RBT positive + 25% of RBT test negative serum

Q-fever
ELISA test-25% of total blood serum

Deletion typing
- RD4 deletion typing (abattoir specimens)
- RD9 deletion typing (human specimens)

MTC
Spoligotyping

NTM
Genus typing

Figure 8 2 Sampling and sample flowchart

8.3 Diagnostic techniques and laboratory methods

8.3.1 Tuberculin skin test

The comparative intradermal tuberculin test (CIDT) was performed using both bovine and avian PPD obtained from the Veterinary Laboratories Agency, Addlestone, Surrey, United

Kingdom. Two injection sites were taken in the middle third of the side of the neck, one above the other, separated at least 12 cm for cattle and camels, while injection sites were taken on both sides of the neck in goats. The hair was shaved around the sites to a radius of about 2 centimeters. Skin fold at both sites were measured with a caliper and the measurements recorded. An aliquot of tuberculin containing 2,500 IU/0.1 ml bovine PPD was injected into the skin intradermally at the lower injection site and similarly tuberculin containing 2,500 IU/0.1 ml avian PPD injected at the upper site for cattle and camels, and for goats avian PPD on the right and bovine PPD on left side of the neck. After 72 hours, the thickness of the same skin fold at both sites was measured and recorded. Bovine and avian positive reactors were obtained using the formula: $[(Bov_{72} - Bov_0)-(AV_{72}-Av_0)]$ and $[AV_{72}-Av_0)-(Bov_{72} - Bov_0)]$, respectively. Bov_0 and Av_0 indicated skin thickness before injecting bovine and avian tuberculin and Av_{72} and Bov_{72} were the corresponding skin fold thickness 72 hours post-injection. The tuberculin test results were interpreted based on OIE recommended cut-off >4 mm and recently suggested 2 mm cut-off [70-72].

8.3.2 Serological tests for the diagnosis of brucellosis and Q-fever

All sera were initially tested by Rose Bengal plate Test (RBT). The same dilutions of serum and antigen (30µ:30µ) was used for all tested animal species. All RBTP positive and randomly selected 25 % of negative sera were further tested using indirect enzyme-linked immunosorbent assay (ELISA) for brucellosis. For RBT 30µ of serum and 30µ of antigen (Rose Bengal stained *B. abortus* antigen obtained from BIO-RAD, Marnes-la-Coquette, France) were mixed and rotated on a glass plate for 4 min. Sera with no visible agglutination were recorded as negative while sera showing agglutination were considered positive. For further analysis RBT positive and randomly selected negative sera, antibody ELISA kits for *Brucella abortus* and *Coxiella brunetti* were obtained from IDEXX Switzerland AG, Stationsstrasse 12, and 3097 Liebefeld-Bern, Switzerland. The test was performed according to manufacturer's instruction. ELISA kit was also used to test 25% of randomly selected sera for Q-fever (see detail in chapter 12).

8.3.3 Cultures and molecular typing

After informed consent was obtained, sputum samples from suspect pulmonary TB patients and FNA samples from suspect TB lymphadenitis patients were collected by trained

laboratory technician or physician. FNA specimens were collected and stored in cryo-tubes with phosphate buffer saline pH 7.2 (PBS) and sputum specimens were collected in universal containers. Suspected tuberculous lesions were collected by trained meat inspectors from cattle slaughtered at Negelle abattoir, and from camels and goats slaughtered at Filtu slaughterhouse. Similarly, sampling of suspected tuberculous lesions was also performed at Mojo and Addis Ababa abattoirs, from slaughtered camels and goats which origin could be traced back to the pastoral areas in southeast Ethiopia. All animal specimens were preserved in PBS in universal containers. All human and animal specimens were stored at 4^{o}C until transported on ice within five days to Armauer Hansen Research Institute (AHRI) laboratory in Addis Ababa. In case of logistic limitations, samples were kept at -20 ^{o}C before transport and further processing at AHRI.

All specimens were processed according to standard methods. Lesioned animal tissue samples were dissected and manually homogenized, then decontaminated with 4% NaOH for 15min and concentrated by centrifugation at 3,000 rpm for 15min. The sediment was neutralized with 2N HCl, using phenol red as an indicator and used to inoculate three different media slants: Two Löwenstein–Jensen (LJ) media supplemented with either glycerol or pyruvate, and Middlebrook 7H11 media [83]. The slants were incubated at 37^{o}C for eight weeks and examined daily for the first week and then weekly for the presence of mycobacterial colonies. Cultures were considered negative if no visible growth was detected after eight weeks of incubation. Microscopic examination of cultures using the Ziehl–Neelsen staining method was performed to detect presence of acid-fast positive bacilli (AFB). AFB positive cultures were prepared as 20% glycerol stocks and stored at –80°C for reference.

Heat-killed cells of each AFB isolate were prepared by mixing ~2 loopfuls of cells (≥ 20µl cell pellet) in 200µl dH_2O followed by incubation at 80°C for 1 hour. Heat-killed AFB samples were used as templates in multiplex polymerase chain reactions (PCR) for typing of *Mycobacterium* genus and Region of Difference (RD; deletion typing), according to protocols previously described [83]. Isolate characterized as NTM was sequenced at the 16S rDNA locus and the sequence used in Basic Local Assignment Search Tool (BLAST) searches of databases at the National Center for Biotechnology Information (NCBI) and Ribosomal Differentiation of Microorganisms (RIDOM) (http://rdna.ridom.de) for further

identification of species [83]. DNA sequencing was performed at Animal Health and Veterinary Laboratories Agency (AHVLA), United Kingdom, using an Applied Biosystems model 3730 automated capillary DNA sequencer. Isolates genetically identified by deletion typing as of the *M. tuberculosis* complex (MTC) were spoligotyped for further strain characterization, as previously described [111]. Spoligotyping data were compared with the Spoligo-International-Typing (SIT) database (http://www.pasteur-guadeloupe.fr:8081/SITVITDemo/ and http://www.cs.rpi.edu/~bennek/tbinsight/tblineage.html) to match SIT numbers and lineage classifications. Isolates identified as *M. bovis* were compared with spoligotype patterns in the international *M. bovis* database (www.mbovis.org). Spoligotype patterns were analysed using spolTools (http://www.emi.unsw.edu.au/spolTools) [112].

8.4 Structure of the Thesis

This thesis presented the results of present study in four chapters as follow:

1. Prevalence of bovine tuberculosis in pastoral cattle herds in the Oromia region, southern Ethiopia.

In this paper, a cross-sectional study of bovine tuberculosis (BTB) was conducted in pastoral cattle herds in south Ethiopia, using the comparative intradermal tuberculin test. The prevalence of BTB and the risk factors for having positive reactor herds were assessed in four pastoral associations (PAs) in two districts of Oromia region, namely Goro-Dola and Liben districts of Guji zone.

2. Low prevalence of bovine tuberculosis in Somali pastoral livestock, South-East Ethiopia.

A cross-sectional study of bovine tuberculosis (BTB) detected by the comparative intradermal tuberculin test was conducted in livestock of the Somali region in four pastoral associations (PAs) in 94 herds of cattle, camels and goats.

3. Zoonotic transmission of tuberculosis between pastoralists and their livestock in South-East Ethiopia.

This study was conducted to investigate the presence of *M. bovis* among human TB patients and characterize *M. tuberculosis* strains and mycobacterial species in livestock and humans in Ethiopian pastoralists of Oromia and Somali Regional States

4. Sero-prevalence of brucellosis and Q- fever in South-East Ethiopian pastoral livestock

A cross-sectional study was conducted in selected pastoral districts of Somali and Oromia Regional States in southeast Ethiopia. The main objective of this study was to determine sero-prevalence of brucellosis and Q-fever in three livestock species (cattle, camels and goats). Eight pastoral associations (PAs) from the selected districts were included in the study. Sera from cattle, camels and goats were screened initially with Rose Bengal plate test (RBT) for brucellosis. All RBT positive and randomly selected negative animals were further tested using i-ELISA tested. Sera from randomly selected cattle, camels and goats were also tested for Q-fever using i-ELISA.

Reference

1. Schelling, E. Human and animal health in nomadic pastoralist communities of Chad: zoonoses, morbidity and health services. (2002).
2. Scoones, I. *Living with uncertainty: new directions in pastoral development in Africa*. (Intermediate Technology Publications: London, 1994).
3. Zinsstag, J., Ould Taleb, M. & Craig, P.S. Editorial: Health of nomadic pastoralists: new approaches towards equity effectiveness. *Trop Med Int Health* **11**, 565-568 (2006).
4. ALIVE Investing in Maintaining Mobility in Pastoral Systemsof the Arid and Semi-Arid Regions of Sub-Saharan Africa: An ALive Policy Note. *Partnership for livestock development, poverty alleviation and sustainable growth* (2010).
5. PFE *Pastoralist perspectives of poverty reduction strategy program of Ethiopia: experiences and lesson from Afar Region of Ethiopia*. (Pastoralist Forum Ethiopia.: Addis Ababa, Ethiopia, 2009).
6. Coppock, D.L. *The Borana Plateau of southern Ethiopia: synthesis of pastoral research, development, and change, 1980-91*. (ILRI (aka ILCA and ILRAD): 1994).
7. Teka, H. Pastoralism and Agro-pastoralism. *Proceeding of the 18th Annual Conference of the Ethiopian Veterinary Association* 107-116 (2004).
8. Hatfield, R. & Davies, J. *Global Review of the Economics ofPastoralism*. (Nairobi, Kenya, 2006).
9. Scoones, I. & Graham,, O. New directions for pastoral development in Africa. *Development in practice* **4**, 188-198 (1994).

10. Abdulahi, M. PASTORAL DEVELOPMENT STRATEGIES / POLICIES IN ETHIOPIA: ACRITICAL ANALYSIS AND EVALUATION. *Third National Conference on Pastoral Development* (2004).
11. Schelling, E. et al. Morbidity and nutrition patterns of three nomadic pastoralist communities of Chad. *Acta Trop* **95**, 16-25 (2005).
12. Brocklesby, M., Hobley, M. & Scott-Villiers, P. *Raising Voice – Securing a Livelihood: The Role of Diverse Voicesin Developing Secure Livelihoods in Pastoralist Areas in Ethiopia*. (Institute of Development Studies: UK, 2010).
13. Schwabe, C.W. *Veterinary Medicine and Human Health*. (Williams and Wilkins: Baltimore, 1984).
14. Zinsstag, J., Schelling, E., Wyss, K. & Mahamat, M.B. Potential of cooperation between human and animal health to strengthen health systems. *Lancet* **366**, 2142-5 (2005).
15. Zinsstag, J., Schelling, E., Waltner-Toews, D. & Tanner, M. From "one medicine" to "one health" and systemic approaches to health and well-being. *Prev. Vet. Med* (2010).doi:10.1016/j.prevetmed.2010.07.003
16. Roth, F. et al. Human health benefits from livestock vaccination for brucellosis: case study. *Bull World Health Organ* **81**, 867-876 (2003).
17. Schelling, E. et al. Human and Animal Vaccination Delivery to Remote Nomadic Families, Chad. *Emerg Infect Dis* **13**, 373-379 (2007).
18. Zinsstag, J. et al. Human Benefits of Animal Interventions for Zoonosis Control. *Emerg Infect Dis* **13**, 527-531 (2007).
19. AHINTC A three years National Strategic Preparedness and Response plan for the Avian human influenza Pandemic threat to Ethiopia. (2006).
20. Katoch, V.K. Infections due to non-tuberculous mycobacteria (NTM). *Indian J Med Res* **120**, 290-304 (2004).
21. Koh, W.-J., Kwon, O.J. & Lee, K.S. Diagnosis and treatment of nontuberculous mycobacterial pulmonary diseases: a Korean perspective. *J. Korean Med. Sci* **20**, 913-925 (2005).

22. Böddinghaus, B., Rogall, T., Flohr, T., Blöcker, H. & Böttger, E.C. Detection and identification of mycobacteria by amplification of rRNA. *J Clin Microbiol.* **28**, 1751–1759 (1990).
23. Sreevatsan, S. et al. Restricted structural gene polymorphism in the Mycobacterium tuberculosis complex indicates evolutionarily recent global dissemination. *Proc Natl Acad Sci U S A.* **94**, 9869–9874 (1997).
24. Brosch, R. et al. A new evolutionary scenario for the Mycobacterium tuberculosis complex. *Proc Natl Acad Sci U S A.* **99**, 3684–3689 (2002).
25. Niemann, S., Richter, E. & Rüsch-Gerdes, S. Differentiation among Members of the Mycobacterium tuberculosis Complex by Molecular and Biochemical Features: Evidence for Two Pyrazinamide-Susceptible Subtypes of M. bovis. *J Clin Microbiol.* **38**, 152–157 (2000).
26. Mostowy, S. et al. Revisiting the Evolution of Mycobacterium bovis. *J Bacteriol.* **187**, 6386–6395 (2005).
27. van Soolingen, D. et al. A novel pathogenic taxon of the Mycobacterium tuberculosis complex, Canetti: characterization of an exceptional isolate from Africa. *Int J Syst Bacteriol* **47**, 1236-45 (1997).
28. Niemann, S. et al. Mycobacterium africanum Subtype II Is Associated with Two Distinct Genotypes and Is a Major Cause of Human Tuberculosis in Kampala, Uganda. *J Clin Microbiol.* **40**, 3398–3405 (2002).
29. Djelouadji, Z., Raoult, D., Daffé, M. & Drancourt, M. A Single-Step Sequencing Method for the Identification of Mycobacterium tuberculosis Complex Species. *PLoS Negl Trop Dis.* **2**, e253 (2008).
30. Aranaz, A. et al. Mycobacterium tuberculosis subsp. caprae subsp. nov.: a taxonomic study of a new member of the Mycobacterium tuberculosis complex isolated from goats in Spain. *Int J Syst Bacteriol* **49 Pt 3**, 1263-73 (1999).
31. Aranaz, A., Cousins, D., Mateos, A. & Dominguez, L. Elevation of Mycobacterium tuberculosis subsp. caprae Aranaz et al. 1999 to species rank as Mycobacterium caprae comb. nov., sp. nov. *Int J Syst Evol Microbiol* **53**, 1785-1789 (2003).

32. Cousins, D.V. et al. Tuberculosis in seals caused by a novel member of the Mycobacterium tuberculosis complex: Mycobacterium pinnipedii sp. nov. *Int J Syst Evol Microbiol* **53**, 1305-1314 (2003).
33. Alexander, K.A. et al. Novel Mycobacterium tuberculosis complex pathogen, M. mungi. *Emerging Infect. Dis* **16**, 1296-1299 (2010).
34. Gordon, S.V., Bottai, D., Simeone, R., Stinear, T.P. & Brosch, R. Pathogenicity in the tubercle bacillus: molecular and evolutionary determinants. *BioEssays* **31**, 378-388 (2009).
35. Gagneux, S. et al. Variable host–pathogen compatibility in Mycobacterium tuberculosis. *Proc Natl Acad Sci U S A* **103**, 2869-2873 (2006).
36. Gutacker, M.M. et al. Single-Nucleotide Polymorphism–Based Population Genetic Analysis of Mycobacterium tuberculosis Strains from 4 Geographic Sites. *Journal of Infectious Diseases* **193**, 121 -128 (2006).
37. Johansen, T.B., Djønne, B., Jensen, M.R. & Olsen, I. Distribution of IS1311 and IS1245 in Mycobacterium avium subspecies revisited. *J. Clin. Microbiol* **43**, 2500-2502 (2005).
38. St Amand, A.L., Frank, D.N., De Groote, M.A. & Pace, N.R. Use of specific rRNA oligonucleotide probes for microscopic detection of Mycobacterium avium complex organisms in tissue. *J. Clin. Microbiol* **43**, 1505-1514 (2005).
39. Biet, F., Boschiroli, M.L., Thorel, M.F. & Guilloteau, L.A. Zoonotic aspects of Mycobacterium bovis and Mycobacterium avium-intracellulare complex (MAC). *Vet Res* **36**, 411-36 (2005).
40. Turenne, C.Y., Wallace, R. & Behr, M.A. Mycobacterium avium in the postgenomic era. *Clin. Microbiol. Rev* **20**, 205-229 (2007).
41. Chao, S.S., Loh, K.S., Tan, K.K. & Chong, S.M. Tuberculous and nontuberculous cervical lymphadenitis: a clinical review. *Otolaryngol Head Neck Surg* **126**, 176-179 (2002).
42. Greenstein, R.J. & Collins, M.T. Emerging pathogens: is Mycobacterium avium subspecies paratuberculosis zoonotic? *Lancet* **364**, 396-397 (2004).

43. September, S.M., Brözel, V.S. & Venter, S.N. Diversity of nontuberculoid Mycobacterium species in biofilms of urban and semiurban drinking water distribution systems. *Appl. Environ. Microbiol* **70**, 7571-7573 (2004).
44. Parrish, S., Myers, J. & Lazarus, A. Nontuberculous Mycobacterial Pulmonary Infections in Non-HIV Patients. *Postgrad Med* **120**, 78 (2008).
45. Zelazny, A.M., Calhoun, L.B., Li, L., Shea, Y.R. & Fischer, S.H. Identification of Mycobacterium species by secA1 sequences. *J. Clin. Microbiol* **43**, 1051-1058 (2005).
46. WHO *Profiles of high-burden countries*. 104-108 (World Health Organization: Geneva, Switzerland, 2008).
47. WHO *GlobalTuberculosisControl2010*. 218 (Global Tuberculosis Control 2010: Geneva, Switzerland, 2010).
48. MOH STRATEGIC PLANTUBERCULOSIS, LEPROSY, TB/HIV AND MDR-TBPREVENTION AND CONTROL:(2010/11 – 2014/15). (2010).
49. FMOH *Tuberculosis, Leprosy and TB/HIV Prevention and Control Programme*. (Addis Ababa, Ethiopia, 2008).
50. Meeussen, A. *A Short Term Consultancy on Tuberculosis Prevention and Control at Community level in Ethiopia*. 23 (German Leprosy and Tuberculosis Relief Association and Federal Ministry of Health of Ethiopia: Addis Ababa, Ethiopia, 2010).
51. Smith, N.H., Gordon, S.V., de la Rua-Domenech, R., Clifton-Hadley, R.S. & Hewinson, R.G. Bottlenecks and broomsticks: the molecular evolution of Mycobacterium bovis. *Nat Rev Micro* **4**, 670-681 (2006).
52. Smith, N.H. et al. Ecotypes of the Mycobacterium tuberculosis complex. *J. Theor. Biol* **239**, 220-225 (2006).
53. Müller, B. et al. African 1, an Epidemiologically Important Clonal Complex of Mycobacterium bovis Dominant in Mali, Nigeria, Cameroon, and Chad. *J Bacteriol* **191**, 1951-1960 (2009).
54. Berg, S. et al. African 2, a clonal complex of Mycobacterium bovis epidemiologically important in East Africa. *J. Bacteriol* **193**, 670-678 (2011).

55. Smith, N.H. et al. European 1: A globally important clonal complex of Mycobacterium bovis. *Infection, Genetics and Evolution* **In Press, Corrected Proof**, (2011).
56. Cosivi, O. et al. Zoonotic tuberculosis due to Mycobacterium bovis in developing countries. *Emerg Infect Dis* **4**, 59-70 (1998).
57. CFSPH Bovine Tuberculosis. (2007).
58. de Lisle, G.W., Mackintosh, C.G. & Bengis, R.G. Mycobacterium bovis in free-living and captive wildlife, including farmed deer. *Rev. - Off. Int. Epizoot* **20**, 86-111 (2001).
59. de Lisle, G.W., Bengis, R.G., Schmitt, S.M. & O'Brien, D.J. Tuberculosis in free-rangingwildlife: detection, diagnosis and management. *Rev. sci. tech. Off. int. Epiz.,* **21**, 317–334 (2002).
60. Wilkins, M.J. et al. Human Mycobacterium bovis Infectionand Bovine Tuberculosis Outbreak,Michigan, 1994–2007. *Emerging Infectious Diseases* **14**, 657-660 (2008).
61. Ayele, W.Y., Neill, S.D., Zinsstag, J., Weiss, M.G. & Pavlik, I. Bovine tuberculosis: an old disease but a new threat to Africa. *Int J Tuberc Lung Dis* **8**, 924-37 (2004).
62. Rodwell, T.C. et al. Prevalence of bovine tuberculosis in African buffalo at Kruger National Park. *J. Wildl. Dis* **37**, 258-264 (2001).
63. Michel, A.L., Klerk, L.-M. de, Pittius, N.C.G. van, Warren, R.M. & Helden, P.D. van Bovine tuberculosis in African buffaloes: observations regarding Mycobacterium bovis shedding into water and exposure to environmental mycobacteria. *BMC Vet Res.* **3**, 23 (2007).
64. Tschopp, R. et al. Bovine tuberculosis in Ethiopian wildlife. *J. Wildl. Dis* **46**, 753-762 (2010).
65. Pollock, J.M. & Neill, S.D. Mycobacterium bovis infection and tuberculosis in cattle. *Vet. J* **163**, 115-127 (2002).
66. Neill, S.D., Pollock, J.M., Bryson, D.B. & Hanna, J. Pathogenesis of Mycobacterium bovis infection in cattle. *Vet. Microbiol* **40**, 41-52 (1994).
67. Whipple, D.L., Bolin, C.A. & Miller, J.M. Distribution of lesions in cattle infected with Mycobacterium bovis. *J. Vet. Diagn. Invest* **8**, 351-354 (1996).
68. O'Reilly, L.M. & Daborn, C.J. The epidemiology of Mycobacterium bovis infections in animals and man: a review. *Tuber Lung Dis* **76 Suppl 1**, 1-46 (1995).

69. OIE Office International des Epizooties Terrestrial manual:World Health Organization for Animal Health,Paris. *Office International des Epizooties* (2009).
70. Ameni, G., Hewinson, G., Aseffa, A., Young, D. & Vordermeier, M. Appraisal of Interpretation Criteria for the Comparative Intradermal Tuberculin Test for Diagnosis of Tuberculosis in Cattle in Central Ethiopia. *Clin. Vaccine Immunol.* **15**, 1272-1276 (2008).
71. Ngandolo, B.N.R. et al. Comparative assessment of fluorescence polarization and tuberculin skin testing for the diagnosis of bovine tuberculosis in Chadian cattle. *Preventive Veterinary Medicine* **89**, 81-89 (2009).
72. Müller, B. et al. Bayesian Receiver Operating Characteristic Estimation of Multiple Tests for Diagnosis of Bovine Tuberculosis in Chadian Cattle. *PLoS ONE* **4**, e8215 (2009).
73. Buddle, B.M., Ryan, T.J., Pollock, J.M., Andersen, P. & de Lisle, G.W. Use of ESAT-6 in the interferon-[gamma] test for diagnosis of bovine tuberculosis following skin testing. *Veterinary Microbiology* **80**, 37-46 (2001).
74. Silva, E. Evaluation of an enzyme-linked immunosorbent assay in the diagnosis of bovine tuberculosis. *Veterinary Microbiology* **78**, 111-117 (2001).
75. Palmer, M.V. & Waters, W.R. Advances in bovine tuberculosis diagnosis and pathogenesis: What policy makers need to know. *Veterinary Microbiology* **112**, 181-190 (2006).
76. Zinsstag, J., Schelling, E., Roth, F. & KAZWALA, R. Economics of Bovine Tuberculosis. *Mycobacterium bovis infection in animals and humans* 360 (2006).
77. Idigbe, E.O., Anyiwo, C.E. & Onwujekwe, D.I. Human pulmonary infections with bovine and atypical mycobacteria in Lagos, Nigeria. *J Trop Med Hyg* **89**, 143-148 (1986).
78. Addo, K. et al. Mycobacterial Species Causing Pulmonary Tuberculosis at the Korle Bu Teaching Hospital, Accra, Ghana. *Ghana Med J* **41**, 52-57 (2007).
79. OLOYA, J. et al. Mycobacteria causing human cervical lymphadenitis in pastoral communities in the Karamoja region of Uganda. *Epidemiol Infect* **136**, 636-643 (2008).

80. Godreuil, S. et al. Intrafamilial Cluster of Pulmonary Tuberculosis Due to Mycobacterium bovis of the African 1 Clonal Complex. *J. Clin. Microbiol.* **48**, 4680-4683 (2010).
81. Michel, A.L., Müller, B. & van Helden, P.D. Mycobacterium bovis at the animal-human interface: a problem, or not? *Vet. Microbiol* **140**, 371-381 (2010).
82. Shitaye, J.E., Tsegaye, W. & Pavlik, I. Bovine tuberculosis infection in animal and humanpopulations in Ethiopia: a review. *Veterinarni Medicina* **52**, 317–332 (2007).
83. Berg, S. et al. The Burden of Mycobacterial Disease in Ethiopian Cattle: Implications for Public Health. *PLoS ONE* **4**, e5068 (2009).
84. Demelash, B. et al. Prevalence of Bovine tuberculosis in Ethiopian slaughter cattle based on post-mortem examination. *Tropical Animal Health and Production* **41**, 755-765 (2009).
85. Regassa, A. et al. Erratum to: A cross-sectional study on bovine tuberculosis in Hawassa town and its surroundings, Southern Ethiopia. *Tropical Animal Health and Production* **42**, 1039 (2010).
86. Tschopp, R. et al. Repeated cross-sectional skin testing for bovine tuberculosis in cattle kept in a traditional husbandry system in Ethiopia. *Vet. Rec* **167**, 250-256 (2010).
87. Mamo, G., Kassaye, A., Sanni, M. & Ameni, G. A cross sectional study of camel tuberculosis in Ethiopia. *Bulletin of Animal Health and Production in Africa* **57**, (2009).
88. Hiko, A. & Agga, G.E. First-time detection of mycobacterium species from goats in Ethiopia. *Trop Anim Health Prod* **43**, 133-139 (2011).
89. Tschopp, R. et al. Bovine tuberculosis at the wildlife-livestock-human interface in Hamer Woreda, South Omo, Southern Ethiopia. *PLoS ONE* **5**, e12205 (2010).
90. CVM The Epidemiology of M. bovis infections in animals and man: A Review. *International Animal Health News,Christian Veterinary Mission* **31**, 1-9 (2008).
91. Zinsstag, J., Schelling, E., Solera, X., Blasco, J. & Moriyon, I. Brucellosis. *Oxford Textbook of Zoonoses* 54-64 (2011).
92. Boschiroli, M.L., Foulongne, V. & O'Callaghan, D. Brucellosis: a worldwide zoonosis. *Current Opinion in Microbiology* **4**, 58-64 (2001).

93. Skalsky, K. et al. Treatment of human brucellosis: systematic review and meta-analysis of randomised controlled trials. *BMJ* **336**, 701 -704 (2008).
94. Seleem, M.N., Boyle, S.M. & Sriranganathan, N. Brucellosis: a re-emerging zoonosis. *Vet. Microbiol* **140**, 392-398 (2010).
95. McDermott, J.J. & Arimi, S.M. Brucellosis in sub-Saharan Africa: epidemiology, control and impact. *Veterinary Microbiology* **90**, 111-134 (2002).
96. Teshome, H., Molla, B. & Tibbo, M. A Seroprevalence Study of Camel Brucellosis in Three Camel-rearing Regions of Ethiopia. *Tropical Animal Health and Production* **35**, 381-390 (2003).
97. Berhe, G., Belihu, K. & Asfaw, Y. Seroepidemiological Investigation ofBovine Brucellosis in the ExtensiveCattle Production System of TigrayRegion of Ethiopia. *International Journal of Applied Research in Veterinary Medicine* **5**, 65-71 (2007).
98. Asmare, K., Asfaw, Y., Gelaye, E. & Ayelet, G. Brucellosis in extensive management system of Zebucattle in Sidama Zone, Southern Ethiopia. *African Journal of Agricultural Research* **5**, 252-263 (2010).
99. Megersa, B. et al. Seroprevalence of brucellosis and its contribution to abortion in cattle, camel, and goat kept under pastoral management in Borana, Ethiopia. *Trop Anim Health Prod* **43**, 651-656 (2010).
100. Regassa, G. et al. Human Brucellosis in Traditional Pastoral Communities in Ethiopia. *International Journal of Tropical Medicine* **4**, 59-64 (2009).
101. Kaabia, N. & Letaief, A. La fièvre Q en Tunisie. *Pathologie Biologie* **57**, 439-443 (2009).
102. Schelling, E. et al. Brucellosis and Q-fever seroprevalences of nomadic pastoralists and their livestock in Chad. *Preventive Veterinary Medicine* **61**, 279-293 (2003).
103. Kelly, P.J., Matthewman, L.A., Mason, P.R. & Raoult, D. Q fever in Zimbabwe. A review of the disease and the results of a serosurvey of humans, cattle, goats and dogs. *S. Afr. Med. J* **83**, 21-25 (1993).
104. Steinmann, P. et al. Seroprevalence of Q-fever in febrile individuals in Mali. *Tropical Medicine & International Health* **10**, 612-617 (2005).
105. Mazyad, S.A.M. & Hafez, A.O. Q fever (Coxiella burnetii) among man and farm animals in North Sinai, Egypt. *J Egypt Soc Parasitol* **37**, 135-142 (2007).

106. Philip, C.B., Hoogstraal, H., Reiss-Gutfreund, R. & Clifford, C.M. Evidence of rickettsial disease agents in ticks from Ethiopian cattle. *Bull World Health Organ* **35**, 127-131 (1966).
107. Abebe, A. Prevalence of Q fever infection in the Addis Ababa abattoir. *Ethiop. Med. J* **28**, 119-122 (1990).
108. Ameni, G. et al. Mycobacterium tuberculosis infection in grazing cattle in central Ethiopia. *Vet. J* **188**, 359-361 (2011).
109. Beyene, D. et al. Identification and genotyping of the etiological agent of tuberculous lymphadenitis in Ethiopia. *J Infect Dev Ctries* **3**, 412-419 (2009).
110. Ameni, G., Vordermeier, M., Aseffa, A., Young, D. & Hewinson, G. Field evaluation of the efficacy of Mycobacterium bovis bacillus Calmette-Guerin against bovine tuberculosis in neonatal calves in Ethiopia. *Clinical and Vaccine Immunology* **17**, 1533-1538 (2010).
111. Kamerbeek, J. et al. Simultaneous detection and strain differentiation of Mycobacterium tuberculosis for diagnosis and epidemiology. *J Clin Microbiol* **35**, 907-914 (1997).
112. Tang, C., Reyes, J.F., Luciani, F., Francis, A.R. & Tanaka, M.M. spolTools: online utilities for analyzing spoligotypes of the Mycobacterium tuberculosis complex. *Bioinformatics* **24**, 2414 -2415 (2008).

9. Prevalence of bovine tuberculosis in pastoral cattle herds in the Oromia region, southern Ethiopia

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Abstract A cross-sectional study of bovine tuberculosis (BTB) was conducted in pastoral cattle herds in Southern Ethiopia, from February to August 2008 using the comparative intradermal tuberculin test (CIDT). The prevalence of BTB and the risk factors for having positive reactor herds were assessed in four pastoral associations (PAs) in two districts of southern Ethiopia, namely Goro-Dola with 242 cattle in 16 herds, and Liben with 231 cattle in 15 herds. A herd was considered positive if there was at least one reactor animal in a herd. The test results were interpreted based on the Office Internationale des Epizooties (OIE) recommended 4 mm and a recently suggested 2 mm cut-off. The apparent individual animal prevalence of tuberculin reactors was 5.5% (95%CI:=4.0 – 8.0%) and 7.0% (95%CI= 5.0 – 10.0%), whereas the true prevalence estimate was 4.4% (95%CI= 0.8 – 8.0%) and 6.1% (95%CI=2.6 - 9.5%), when using the 4 mm and the 2 mm cut-off, respectively. The overall herd apparent prevalence of tuberculin reactor animals was 41.9% (95% CI= 24.9 - 60.9%) and 48.4% (95%CI:=30.2 - 66.9%) with the 4 mm and 2 mm cut-off, respectively. A positive tuberculin test was associated with the age of animals and the main drinking water sources during dry seasons. In order to investigate the public health risks and the epidemiological importance of BTB in the area, we recommend to include other livestock species (camels and goats) as well as humans in future studies.

Keywords: prevalence; bovine tuberculosis; pastoralist; Oromia; south Ethiopia

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Introduction

Tuberculosis in mammals is caused by mycobacteria belonging to the *Mycobacterium tuberculosis* complex (MTC), with *Mycobacterium tuberculosis* being the most common cause of tuberculosis in humans, whereas BTB is caused by *Mycobacterium bovis* (Inangolet et al. 2008) and a chronic bacterial disease of animals and humans and is a major infectious disease among cattle that are considered to be the main host of *Mycobacterium bovis (M. bovis).* Aerosol exposure to *M. bovis* is considered to be the most frequent route of infection (Biet et al. 2005). The standard method for BTB detection in live cattle is usually by the comparative intradermal test (CIDT) based on delayed hypersensitivity reactions. OIE recommends the cut-off point of greater than 4 mm for skin test positivity (OIE 2008). However, recently other authors suggested 2 mm cut-off as being more adequate for Africa (Ameni et al. 2008; Bongo et al. 2009).

Following the introduction of milk pasteurization as well as large scale test and slaughter programs in cattle, the occurrence of BTB became rare in humans and cattle in industrialized countries (Cosivi et al. 1998; Ayele et al. 2004). However, it remains an important disease in many countries of the world where BTB is endemic, causing significant economic losses (Zinsstag et al. 2006). BTB in animals has been reported from 33 of 43 African countries (Ayele et al. 2004) and human BTB cases have so far not been described in Sahelian countries like Burkina Faso, Niger and Chad (Rey et al. 1982; Diguimbaye et al. 2006; Godreuil et al. 2007), but have been described in Ghana, Nigeria, Uganda and Tanzania (Idigbe et al. 1986; Addo et al. 2007; Oloya et al.2008).

In central Ethiopia, BTB is endemic in cattle with varying prevalence of 3.5-5.2% in abattoir and 3.5-50.0% in cross-breeds dairy farms (Shitaye et al. 2007; Berg et al. 2009; Demelash et al. 2009; Regassa et al 2010). Tuberculin skin test survey conducted in the three districts among zebu cattle kept traditionally were reported an overall prevalence of 0.9% applying the OIE definition and 3.0% using a 2 mm cut-off (Tschopp et al.2009; Tschopp et al. 2010). *M. bovis* was also isolated from milk of BTB reactors in dairy farms in and around Addis Ababa, highlighting the public health importance of the disease (Bogale and Lemma 2004; Ameni and Erkihun 2007). Information on BTB among pastoral cattle herds is scarce in Ethiopia and the disease situation is not known in the pastoral settings. Therefore, the objectives of the present study were to assess the

prevalence of tuberculin reactors in pastoral herds in southern Ethiopia and to evaluate the determinants of tuberculin reacting cattle.

Materials and methods

Study Area

The study was conducted from February to August 2008 in Liben and Goro-Dola pastoral districts of Guji zone in the Oromia regional state in southern Ethiopia. The two districts were situated between the 39.09-40.03° E longitudes and 4.52-5.5° N latitude. Climatic condition of the area was characterized by arid and semi-arid lowland weather with bimodal rainfall pattern. The pastoralist communities in the area were keeping local zebu breeds, composed of Boran and Guji cattle or cross-breeds of the two. Livestock husbandry was identical for both districts with all pastoralists keeping their animals extensively.

Selection of pastoral associations

Considering Negelle town (Zonal capital) (Figure 8.1) as reference, four PAs were selected for this study that were located geographically on the four directions and in a distance of 30-40 km from Negelle, namely Dhuko PA in the west, Sirba PA in the north, Arda-Bururi PA in the east and Siminto PA in the south.

Sample size

The sample size for tuberculin testing of cattle was calculated using the cluster sampling formula provided by Bennett et al. (1991).We assumed an intraclass correlation coefficient (rho) of 0.2, an expected prevalence of 3.0% and a standard error of 1.5%. The total sample size calculated for 32 herds was n=480 cattle; however the tested cattle were 473 in 31 herds.

$$\rho = \frac{(withinHerdVariation)}{(Total\ \mathrm{var}\, iation)}$$

$$D = 1 + (b-1)\rho$$

$$s.e._x = \sqrt{\frac{pqD}{n}} = \sqrt{\frac{pqD}{cb}}$$

ρ=roh; se = standard error; D = design effect; b = samples per clusters; c= number of cluster; p=prevalence (Bennett et al. 1991).

A total of 122, 120, 120 and 111 animals were tested in Dhuko, Sirba, Arda-Bururi and Siminto PAs, respectively. A list of registered households from each PA that were interested to participate in the study was used as a sampling frame following agreement on procedures to be followed during a general PA meeting on each site. Eight households were randomly selected from the list for each PA. Fifteen animals with age of greater or equal to one year were randomly selected per herd and temporary unique identification numbers were given for each tested animal together with sex, age, breed and body condition score. Breeds were classified as Boran (b), Guji cattle (g) or cross breeds of the two (gb) based on breed phenotypical characteristics. Cattle were categorized into following age group: young (1-2.5 years), adult (2.6-6 years) and old (>6 years). Body condition in individual animals was assessed using a modified guideline described by Msangi et al (1999) and animals were classified as emaciated (score 1), thin (score 2), normal (score 3), musculous (score 4) and fat (score 5).

Tuberculin skin testing

Comparative intradermal tuberculin test was performed using both bovine and avian mycobacterium purified protein derivative (PPD) obtained from the Veterinary Laboratories Agency, Addlestone, Surrey, United Kingdom. Two injection sites were chosen in the middle third of the side of the neck, one above the other, separated at least by 12 cm. Approximately 2 cm radius hairs around the injection sites were shaved. Skin fold at both sites were measured with a caliper and the measurements recorded. An aliquot of tuberculin containing 2500 IU/0.1 ml bovine PPD was injected intradermally in the lower injection site; similarly 2500 IU/0.1 ml avian PPD was injected in the upper site. After 72 hours, the thickness of the same skin fold at both sites was measured and recorded.

Bovine and avian positive reactors were obtained using the formula: $[(Bov_{72} - Bov_0)-(AV_{72}-Av_0)]$ and $[AV_{72}-Av_0)-(Bov_{72} - Bov_0)]$, respectively. Bov_0 and Av_0 indicated skin thickness before injecting bovine and avian tuberculin, respectively, and Bov_{72} and Av_{72} were the corresponding skin fold thickness 72 hours post-injection. The tuberculin test results were interpreted based on OIE recommended 4 mm cut-off as well as a 2 mm cut-off (Ameni et al. 2008; Bongo et al. 2009). The following criteria for final classification of reactor cattle were used:

A) Using OIE standard cut-off >4 mm

- Increase in skin fold thickness >4 mm was regarded as positive reactor
- Increase in skin fold thickness 1 to 4 mm was regarded as doubtful reactor.
- The reaction was considered to be negative for BTB if the increase in skin thickness at the bovine site of injection was less than the increase in the skin reaction at the avian site of injection
- Increase in skin fold thickness >1 mm at avian site than at the bovine site was considered as positive for *Mycobacterium avium spp*.
- Reaction was considered as equal reactor if increase in skin thickness at the both sites were equal.

B) Using cut-off >2 mm

- Increase in skin fold thickness >2 mm was regarded as positive reactor.
- Increase in skin fold thickness 1 to 2 mm was regarded as doubtful reactor.
- The reaction was considered to be negative for BTB if the increase in skin thickness at the bovine site of injection was less than the increase in the skin reaction at the avian site of injection.
- Increase in skin fold thickness >1 mm at avian site than at the bovine site was considered as positive for *M. avium spp*.
- Reaction was considered as equal reactor if increase in skin thickness at the both sites were equal.

Questionnaire survey and focus group discussion

All herd owners of tuberculin tested cattle were interviewed by means of pre-tested questionnaires in order to assess possible risk factors for tuberculin positivity. In addition to the questionnaires, a focus group discussion (FGD) was conducted at each study PA using a semi-structured interview catalogue.

Data entry and analysis

The data was double entered in Microsoft Access 2002 (Microsoft Corp. Redmond, USA) and validated with EpiInfo version 3.3.2 before being imported to Stata 10/SE (Stata Corp., College Station, TX) for analysis.

The outcome of all statistical analyses was individual animal and herd level binary outcomes. A herd was considered positive if it had at least one tuberculin reactor.

Explanatory variables were cross-tabulated using, Pearson's chi-square test or Fisher's exact test if cells with less than 5 of expected frequencies occurred. Logistic regression model with random effect was used for the multivariate analysis in order to account for clustering on the herd level and the following explanatory variables have been added in the model: PA, age group, sex, breed and body condition score (BCS).

Results

Individual animal prevalence

A total of 473 cattle from 31 randomly selected herds were tuberculin tested, 231 animals from Liben and 242 from Goro-Dola districts. The number of tested animals per study PA was 122 from Dhuko, 120 from Sirba, 120 from Adarda-Bururi and 111 from Siminto. Overall apparent individual animal prevalence was 5.5% (95%CI= 4.0 – 8.0%) when using the 4 mm cut-off. There was a significant difference in proportions of positive animals between study PAs (p=0.003) and between age group of animals (p = 0.046) (Table 9.1). Multivariate analysis showed significant differences of CIDT positivity between age groups (p=0.02) and animals older than 6 years of age were at higher risk of infection (OR=13.7, 95% CI=1.5, 122.0) (Table 9.1).

When 2 mm cut-off was used, overall apparent individual animal prevalence was 7.0% (95%CI= 5 – 10.0%) (Table 9.1). There was significant difference in prevalence between study PAs (p<0.001) and between age groups (p=0.03). There was no significant difference between body condition scores, sex and breed types and positive reactors.

The true prevalence was calculated using the following formula described by Rogan and Gladen (1978), $TP = (AP + SP - 1)/(SE + SP - 1)$, where TP is true prevalence, AP the apparent prevalence, SE is sensitivity, and SP is specificity. TP was calculated using 59.4% and 69.0% sensitivity for the 4 mm and 2 mm cut-off respectively, and 97.0% specificity for both cut-offs as described by Ameni et al (2008) for local Zebu cattle in Ethiopia. Accordingly, the true prevalence was 4.4 % (95%CI= 0.8 – 8.0%) for the 4 mm cut-off and 6.1% (95%CI: 2.6 - 9.5%) for the 2 mm cut-off. Overall, 3.4 %(16/473) and 1.9% (9/473) were doubtful reactors with the 4 mm and 2 mm cut-off, respectively. Eight animals (1.7%) were reacting to *M. avium* antigen. Four animals (0.85 %) reacted equally to *M. bovis* and *M. avium* antigens.

Table 9 1 Individual animal prevalence stratified by pastoral association (PA), age group, sex, breed types and body condition scores using the CIDT at a cut off at 4 mm and 2 mm

Risk factors	Number tested	Prevalence Number positives (%)		χ^2/Fisher P-value		Multivariate analysis OR** (95% CI)		P-value	
		>4mm	>2mm	> 4mm	>2mm	>4mm	>2mm	> 4mm	> 2mm
Study sites (PA)									
Sirba	120	5 (4.2)	6 (5.0)			1	1	-	-
Arda-Bururi	120	12 (10.0)	13 (10.8)			4.5 (0.95-21.5)	3.7 (0.87-16.1)	0.06	0.08
Siminto	111	9 (8.1)	14 (12.6)			2.2 (0.40-11.8)	3.3 (0.72-15.6)	0.36	0.13
Dhuko	122	0	0			-	-	-	-
		26 (5.5)	33 (7.0)	0.003	<0.001				
Age group									
Young(1-2.5 years)	105	1 (1.0)	2 (1.9)			1	1	-	-
Adult (2.6-6years)	205	12 (5.9)	14 (6.8)			9.8 (1.1-84.0)	4.6 (0.90 – 22.4)	0.04	0.06
Old (>6years)	163	13 (8.0)	17 (10.4)			13.7 (1.5-122)	6.8 (1.4 -34.3)	0.02	0.02
				<0.05	0.03				
Sex									
Female	342	17(5.0)	22 (6.4)			1	1	-	-
Male	131	9 (6.9)	11(8.4)			2.4 (0.9-6.8)	1.9 (0.8-4.8)	0.09	0.15
				0.42	0.45				
Breed types									
Cross (GB)	80	2(2.5)	4 (5.0)			1	1	-	-
Guji (G)	243	12(4.9)	14 (5.8)			5.1 (0.9-27.5)	2.5 (0.66-9.2)	0.06	0.18
Boran(B)	149	12 (8.1)	15 (10.1)			3.5 (0.7-17.7)	1.7 (0.49-5.9)	0.13	0.40

				0.23	0.20				
BCS*									
Emaciated (1)	0	-	-						
Thin (2)	59	4 (6.8)	6 (10.2)			1	1	-	-
Normal (3)	294	16 (5.4)	20 (6.8)			1.1 (0.3-3.8)	0.9 (0.3-2.6)	0.86	0.86
Musculous (4)	113	6 (5.3)	7 (6.2)			1.0 (0.2-4.7)	0.8 (0.2-3.0)	0.97	0.79
Fat (5)	0	0	0			-	-		-
				0.94	0.76				

* *Body condition sore, ** Odds ratio*

Herd prevalence

Out of 31 tested herds the prevalence was 41.9% (95% CI= 24.9 - 60.9%) and 48.4% (95% CI=30.2 – 66.9%) when using the 4 mm and 2 mm cut-off, respectively. A significant difference was observed between herds drinking water from river and communal stagnant water sources (dam, traditional wells and big communal boreholes) during the main dry season ($p=0.01$ and $p=0.002$) when using the 4 mm and 2 mm cut-off, respectively (Table 9.2). No significant association was found between reactor herds and herd size, recent introduction of new animals into the herd, herd contact frequency, annual herd movement dynamics and keeping of different livestock together.

Table 9 2 Hypothesized risk factors for bovine tuberculosis reactors in 31 cattle herds using the CIDT at a cut-off 4mm and 2mm.

hypothesized risk factors	Positive herds (%) (n=31)		χ^2/Fisher P-value	
	>4mm	>2mm	>4mm	>2mm
Small ruminants within cattle herd	11(35.0)	13(42.0)	0.17	0.23
Goats				
Sheep	9(30.0)	10(32.0)	0.05	0.05
Herd with newly introduced animals	13(42.0)	15(48.0)	0.50	0.49
Herd size				
15-50 cattle	13(42.0)	15(48.0)		
51-100 cattle	-	-		
> 100 cattle	-	-		
			1	1
Cattle herd migration and distance per year				
>30 km	10(32.0)	12(39.0)		
20-30 km	2(6.0)	2(6.0)		
No migration	1 (3.0)	1(3.0)		
			0.32	0.22
Drinking water sources during dry seasons				
River	-	-		
Static water (dams, wells & boreholes)	13(42.0)	15 (48.0)		
			0.006	0.002

Discussion

The apparent BTB prevalence of 5.5% (95%CI=4% - 8%) and 7.0% (95%CI: 5.0% -10.0%) in southern Ethiopia pastoral cattle herds at the cut-off 4 mm and 2 mm, respectively was comparable with other results reported from Ethiopia (Ameni et al. 2003; Fetene and Kebede 2009; Tschopp et al. 2009), Uganda (Bernard et al. 2005) and Zambia (Munyeme et al. 2008). Variable results were reported from central part of Ethiopia and other countries of Africa (Omer et al. 2001; Shirima et al. 2003; Oloya et al. 2006; Ameni et al. 2007; Inangolet et al. 2008; Regassa et al. 2008). Differences in management practices, production system, types of cattle breeds, or different ecological zones as well as cattle trade may explain the seen inter-study variation.

The odds ratio of being a tuberculin reactor for the animals older than 6 years was 13.7 and 6.8 times higher as compared to younger animals at the cut-off 4 mm and 2 mm, respectively. This result was in agreement with the previous studies (Ameni et al. 2007; Inangolet et al. 2008, Regassa et al. 2008) and may be explained by the chronic nature of the disease, and long exposure to the agent over time.

In contrast to other study results (Ameni et al. 2007; Munyeme et al. 2008; Regassa et al. 2008) there was no association between body condition score and positive reactor. In our study, the majority of cattle were in good body condition (score 3 or 4), which may explain the observed variation. Ameni et al. (2007) reported significant variation to tuberculin positivity between Holsteins breed (*Bos taurus*) and local zebu (*Bos indicus*) in Ethiopia. We tested in our study only zebu cattle and there was no association between the different cattle breeds kept by communities in the study PAs and BTB reactors.

According to the OIE guidelines, the 3.4% (4 mm cut-off) and 1.9% (2 mm cut-off) doubtful reactors should have been re-tested after 60 days. This was however, unfeasible, due to the mobility of pastoral herds, logistics and time limitations.

Compared to reports of Fetene and Kebede (2009) from north Ethiopia and Shirima et al. (2003) from Tanzania, a low percentage of reactors to *M. avium* antigen (1.7%) was observed in the present study, which may be due to difference in ecological zones with varying presence of *M. avium* or other non-tuberculous mycobacteria.

In the present study, some animals reacted strongly and equally to both *M. bovis* and *M. avium* antigens. This might be caused either by a non specific reaction to mycobacterial

PPD or by a mixed infection with both *M. bovis* and *M. avium*. However, results can only be confirmed once the pathogens have been isolated.

The observed herd prevalence of 41.9% (4 mm cut-off) and 48.4% (2 mm cut-off), were comparable to previous results in Ethiopia and other African countries (Omer et al. 2001; Ameni et al. 2003; Oloya et al. 2006; Oloya et al. 2007; Regassa et al. 2008; Fetene and Kebede 2009).

We observed significant difference in prevalence between herds' drinking water from river and stagnant water sources during the main dry season, which may be due to aggregation of large number of different livestock from different pastoral households around limited watering points facilitating BTB transmission either directly between animals or by contaminated pastures and water sources. Similar observation was also reported from Uganda (Oloya et al. 2006).

A broad range of hypothesized 'classical' risk factors such as herd size, herd keeping with other livestock species (goats and sheep), contact with other herds and annual migration dynamics, recent introduction of new animals to herd were not associated with herd positivity to BTB. This is probably due to the high similarities in management practices in this particular region.

Despite of observed similar husbandry practices and high mobility of livestock in the study area, the BTB prevalence was high in some study sites, namely; Arda-Bururi and Siminto PAs as hot spot, whereas it was zero in Dhuko PA. We do not have explanation to these variations in prevalence between the study sites and further investigation is needed to assess the role of water sources for livestock and other possible risk factors.

BTB infection causes milk and meat productivity losses to livestock owners in addition to decreased value of the slaughter animal if condemned during meat inspection. Close contact to animals, consumption of raw milk and pooling of un-pasteurized milk from different animals for home consumption and marketing are common practices among pastoral communities in the study area, which entail the risk of zoonotic transmission of tuberculosis. Moreover, the study area has the highest cattle population densities in the country and is a major source of livestock for domestic and export market. Unofficial cross-border livestock trading to the neighboring countries is also common practices in the area.

Knowing the BTB epidemiology in such pastoralist zones is important in regard of the possible public health implications, and the impact of the disease on livelihood and economy of pastoralists. Further studies are necessary in order to assess the role of other livestock, in particular camels and goats kept together with cattle. The prevalence of human tuberculosis caused by *M. bovis* is largely unknown in the area and needs furthers investigation.

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References

Addo, K., Owusu-Darko, K., Yeboah-Manu, D., Caulley, P., Minamikawa, M., Bonsu, F., Leinhardt, C., Akpedonu, P. and Ofori-Adjei, D., 2007. Mycobacterial Species Causing Pulmonary Tuberculosis at the Korle Bu Teaching Hospital, Accra, Ghana. Ghana Medical Journal, 41 (2), 52-57.

Ameni, G., Amenu, K. and Tibbo, M., 2003. Prevalence and Risk Factor Assessment in Cattle and Cattle Owners in Wuchale-Jida District, Central Ethiopia, The International Journal of Applied Research in Veterinary Medicine, 1 (1), 17-25.

Ameni, G. and Erkihun, A., 2007. Bovine tuberculosis on small-scale dairy farms in Adama Town, central Ethiopia, and farmer awareness of the disease, Revue Scientifique Et Technique (International Office of Epizootics), 26 (3), 711-9.

Ameni, G., Aseffa, A., Engers, H., Young, D., Gordon, S., Hewinson, G. and Vordermeier, M., 2007. High Prevalence and Increased Severity of Pathology of Bovine Tuberculosis in Holsteins Compared to Zebu Breeds under Field Cattle Husbandry in Central Ethiopia, Clinical and Vaccine Immunology, 14 (10), 1356–1361.

Ameni, G., Hewinson, G., Aseffa, A., Young, D. and Vordermeier, M., 2008. Appraisal of Interpretation Criteria for the Comparative Intradermal Tuberculin Test for Diagnosis of Tuberculosis in Cattle in Central Ethiopia, Clinical and Vaccine Immunology 15 (8), 1272-1276.

Ayele, W. Y., Neill, S. D., Zinsstag, J., Weiss, M. G. and Pavlik, I., 2004. Bovine tuberculosis: an old disease but a new threat to Africa, The International .Journal of Tuberculosis and Lung Disease, 8, 924-937.

Bennett, S., Woods, T., Liyanage, W.M. and Smith, D.L., 1991. A simplified general method for cluster-sample surveys of health in developing countries, World Health Statistics Quarterly. Rapport Trimestriel De Statistiques Sanitaires Mondiales, 44 (3), 98-106.

Berg, S., Firdessa, R., Habtamu, M., Gadisa, E., Mengistu, A., Yamuah, L., Ameni, G., Vordermeier, M., Robertson, B.D., Smith, N.H., Engers, H., Young, D., Hewinson, R.G., Aseffa, A. and Gordon, S.V., 2009. The Burden of Mycobacterial Disease in Ethiopian Cattle: Implications for Public Health, PLoS ONE, 4 (4), 5068.

Bernard, F., Vincent, C., Matthieu, L., David, R. and James, D., 2005. Tuberculosis and brucellosis prevalence survey on dairy cattle in Mbarara milk basin (Uganda), Preventive Veterinary Medicine, 67 (4), 267-81.

Biet, F., Boschiroli, M.L., Thorel, M.F. and Guilloteau, L.A., 2005. Zoonotic aspects of Mycobacterium bovis and Mycobacterium avium-intracellulare complex (MAC). Veterinary Research, 36 (3), 411-36.

Bogale, A. and Lemma, E., 2004. Isolation of Mycobacteria from raw milk of tuberculin sensitive dairy cows in Addis Ababa, Ethiopian Veterinary Journal, 8 (1), 1-6.

Bongo, N. R. N., Müller, B., Diguimbaye-Djaibe, C., Schiller, I., Marg-Haufe, B., Cagiola, M., Jolley, M., Surujballi, O., Akakpo, A. J., Oesch, B. and Zinsstag, J., 2009. Comparative assessment of Fluorescence Polarisation and tuberculin skin testing for the diagnosis of bovine tuberculosis in Chadian cattle, Preventive Veterinary Medicine, 89, 81-89.

Cosivi, O., Grange, J.M., Daborn, C.J., Raviglione, M.C., Fujikura, T., Cousins, D., Robinson, R.A., Huchzermeyer, H.F., de Kantor, I. and Meslin, F.X., 1998. Zoonotic tuberculosis due to Mycobacterium bovis in developing countries, Emerging Infectious Diseases, 4 (1), 59-70.

Demelash, B., Inangolet, F., Oloya, J., Asseged, B., Badaso, M., Yilkal, A. and Skjerve, E., 2009. Prevalence of Bovine tuberculosis in Ethiopian slaughter cattle based on post-mortem examination, Tropical Animal Health and Production, 41 (5), 755-765.

Diguimbaye, C., Hilty, M., Ngandolo, R., Mahamat, H. H., Pfyffer, G. E., Baggi, F., Tanner, M., Schelling, E. and Zinsstag, J., 2006. Molecular characterization and drug resistance testing of Mycobacterium tuberculosis isolates from Chad, Journal of Clinical .Microbiology, 44**,** 1575-1577.

Fetene, T. and Kebede, N., 2009. Bovine tuberculosis of cattle in three districts of northwestern Ethiopia, Tropical Animal Health and Production, 41 (2), 273-277.

Godreuil, S., Torrea, G., Terru, D., Chevenet, F., Diagbouga, S., Supply, P., Van De, P. P., Carriere, C.and Banuls, A. L., 2007. First molecular epidemiology study of Mycobacterium tuberculosis in Burkina Faso, Journal of Clinical .Microbiology, 45**,** 921-927.

Idigbe, E. O., Anyiwo, C. E. and Onwujekwe, D. I.,1986.Human pulmonary infections with bovine and atypical mycobacteria in Lagos, Nigeria, Journal of Tropical Medicine and Hygiene, 89 (3), 143-148.

Inangolet, F.O., Demelash, B., Oloya, J., Opuda-Asibo, J. and Skjerve, E., 2008. A cross-sectional study of bovine tuberculosis in the transhumant and agro-pastoral cattle herds in the border areas of Katakwi and Moroto districts, Uganda, Tropical Animal Health and Production, 40 (7), 501-8.

Msangi, B.S.J., Bryant, M.J, Kavana, P.Y., Msanga, Y.N. and Kizima, J.B., 1999. Body measurements as a management tool for crossbred dairy cattle at a Smallholder farm condition. In: Proceedings of the 26th Scientific Conference of the Tanzanian Society of Animal Production, Arusha, 1997.

Munyeme, M., Muma, J.B., Skjerve, E., Nambota, A.M., Phiri, I.G.K., Samui, K.L., Dorny, P. and Tryland, M., 2008. Risk factors associated with bovine tuberculosis in traditional cattle of the livestock/wildlife interface areas in the Kafue basin of Zambia, Preventive Veterinary Medicine, 85 (3-4), 317-28.

OIE., 2008. Office International des Epizooties Terrestrial manual: Chapter 2.4.7, World Health Organization for Animal Health

OLoya, J., Opuda-Asibo, J., Kazwal, R., Demelash, A., Skjerve, E., Lund, A., Johansen, T. and Djonne., B., 2008. Mycobacteria causing human cervical lymphadenitis in pastoral communities in the Karamoja region of Uganda. Epidemiology and Infection, 136 (5), 636-643.

Oloya, J., Muma, J.B., Opuda-Asibo, J., Djønne, B., Kazwala, R. and Skjerve, E., 2007. Risk factors for herd-level bovine-tuberculosis seropositivity in transhumant cattle in Uganda, Preventive Veterinary Medicine, 80 (4), 318-29.

Oloya, J., Opuda-Asibo, J., Djønne, B., Muma, J., Matope, G., Kazwala, R. and Skjerve, E,. 2006. Responses to tuberculin among Zebu cattle in the transhumance regions of Karamoja and Nakasongola district of Uganda, Tropical Animal Health and Production, 38 (4), 275-283.

Omer, M.K., Skjerve, E., Woldehiwet, Z. and Holstad, G., 2001. A cross-sectional study of bovine tuberculosis in dairy farms in Asmara, Eritrea, Tropical Animal Health and Production, 33 (4), 295-303.

Regassa, A., Medhin, G. and Ameni, G., 2008. Bovine tuberculosis is more prevalent in cattle owned by farmers with active tuberculosis in central Ethiopia, The Veterinary Journal, 178, 119-125.

Regassa, A., Tassew, A., Amenu, K., Megersa, B., Abunna, F., Mekibib, B., Marcotty, T. and Ameni, G., 2010. Erratum to: A cross-sectional study on bovine tuberculosis in Hawassa town and its surroundings, Southern Ethiopia. Tropical Animal Health and Production, 42 (5), 1039.

Rey, J.L., Pestiaux, J.L., Bichat, B. and Meyer, L., 1982. [Bacteriological study of 174 mycobacterial strains isolated from tuberculosis patients in Niger]. Annales De La Société Belge De Médecine Tropicale, 62 (1), 55-60.

Rogan, W. J. and Gladen, J., 1978. Estimating prevalence from the results of screening test, American Journal of Epidemiology, 107, 71–76.

Shirima, G.M., Kazwala, R.R. and Kambarage, D.M., 2003. Prevalence of bovine tuberculosis in cattle in different farming systems in the eastern zone of Tanzania, Preventive Veterinary Medicine, 57 (3), 167-72.

Shitaye, J., Tsegaye, W. and Pavlik, I., 2007. Bovine tuberculosis infection in animal and human populations in Ethiopia: a review, Veterinarni Medicina, 52 (8), 317–332.

Tschopp, R., Schelling, E., Hattendorf, J., Young, D., Aseffa, A. and Zinsstag, J., 2010. Repeated cross-sectional skin testing for bovine tuberculosis in cattle in traditional husbandry system in Ethiopia, The Veterinary Record, 176, 250-256.

Tschopp, R., Schelling, E., Hattendorf, J., Aseffa, A. and Zinsstag, J., 2009. Risk factors of bovine tuberculosis in cattle in rural livestock production systems of Ethiopia, Preventive Veterinary Medicine, 89 (3-4), 205-211.

Zinsstag, J., Schelling, E., Roth, F. and Kazwala R., 2006. Economics of Bovine Tuberculosis. In: Thoen, C.O., Steele, J.H., Gilsdorf, M.J. (2nd eds), *Mycobacterium bovis* infection in animals and humans, Blackwell, Boston.

10. Low prevalence of bovine tuberculosis in Somali pastoral livestock, South-East Ethiopia

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Abstract A cross-sectional study of bovine tuberculosis (BTB) detected by the comparative intradermal tuberculin test (CIDT) was conducted in livestock of the Somali region in South East Ethiopia - in four pastoral associations (PAs) from January to August 2009. In 94 herds, each fifteen cattle, camels and goats were tested per herd leading to a total of 1418 CIDT tested animals, with 421 cattle, 479 camels and 518 goats. A herd was considered positive if it had at least one reactor. The individual animal prevalence were 2.0% (95% CI=0.5-8.4), 0.4% (95% CI 0.1-3%) and 0.2% (95% CI 0.03-1.3) in cattle, camels and goats, respectively. Prevalence of avian mycobacterium purified protein derivative (PPD) reactors in cattle, camels and goats were 0.7% (95% CI=0.2-2.0%), 10.4 %(95% CI =7.0-14.0 %) and 1.9% (95% CI= 0.9-4.0%), respectively, whereby camels had an odds ratio (OR) of 16.5 (95% 5.0- 55.0) when compared to cattle. There was no significant difference between livestock species in BTB positivity. A range of risk factors included in the present study were not associated with herd positivity to BTB. In the present study the prevalence of bovine tuberculosis was low in Somali pastoral livestock in general and in camels and goats in particular. The high proportion of camel reactors to avian PPD needs further investigation of its impact on camel production.

Keywords: CIDT, bovine tuberculosis, camel, cattle, goats; pastoralist; Somali Region; Ethiopia

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Introduction

Bovine tuberculosis (BTB) is a chronic bacterial disease of animals and humans and is a major infectious disease among cattle, other domesticated animals and certain wildlife populations in a large number of countries (Cosivi et al., 1998; Schiller et al., 2010). Although cattle are considered to be the main hosts of *Mycobacterium bovis* (*M. bovis*), isolations have been made from many other livestock and wildlife species and transmission to humans constitutes a public health problem (Ayele et al., 2004; OIE, 2009). Aerosol exposure to *M. bovis* is considered to be the most frequent route of infection of cattle, but infection by ingestion of contaminated material also occurs (Biet et al., 2005). The standard method for BTB detection in live animal is the comparative intradermal test (CIDT) based on delayed hypersensitivity reactions. The CIDT test includes bovine and avian tuberculin and is used mainly to differentiate between animals infected with *M. bovis* and those sensitized to tuberculin due to exposure to other mycobacteria or related genera (OIE, 2009).

The occurrence of BTB became rare in humans and cattle in industrialized countries (Cosivi et al., 1998; Ayele et al., 2004). However, it remains an important disease in many countries of the world where BTB is endemic, causing significant economic losses (Zinsstag et al., 2006). BTB in animals has been reported from 33 of 43 African countries (Ayele et al., 2004). Human bovine tuberculosis cases have been described in some Sahelian countries like, Ghana, Niger, Uganda and Tanzania (Idigbe et al. 1986; Addo et al. 2007; Oloya et al.2008) and in immigrants from Chad to France (Godreuil et al., 2010). The representative proportion of BTB in human tuberculosis is estimated at less than 5% worldwide (Cosivi et al., 1998; Michel et al., 2010). In Ethiopia, BTB is endemic in cattle. Prevalence varies from 3.5-50.0% depending on the geographical areas, the breeds and the husbandry practices (Shitaye et al., 2007; Berg et al., 2009; Demelash et al., 2009; Regassa et al., 2010). Prevalence in traditionally kept zebu cattle varies between 0.9-4.0% based on different cut-off values used for interpretation (Tschopp et al., 2010). Gumi et al 2011 reported 4.4% individual prevalence in cattle of Oromia pastoralist in Guji zon. Based on gross pathology prevalence of 5.0-10.0% were reported in camels slaughtered at Dire Dawa abattoir in eastern Ethiopia and in Addis Ababa abattoir(Mamo et al., 2009; Mamo et al.,

2011). Hiko and Agga (2011) reported 4.2% prevalence of bovine TB in goats slaughtered at Mojo export abattoir in central Ethiopia base on gross lesions.

Up to date, there are no reports on the CIDT status in Somali pastoral livestock in South-East Ethiopia and information on BTB among the pastoral livestock is generally limited. Therefore, the objective of the present study was to assess the prevalence of tuberculin reactors in the Somali pastoral livestock in south-east Ethiopia and to evaluate the determinants of tuberculin reacting animals.

Materials and methods

Study Area

A cross-sectional cluster sampling study was conducted from January to August 2009 in Filtu Woreda of Liben zone in the Somali regional state in the South-East Ethiopia (Figure10.1). Climatic condition of the area was characterized by arid weather with bimodal rainfall pattern. Considering Filtu town (Zonal capital) as reference center four PAs were selected for this study that located geographically on the three directions except Bakaka PA and in a distance of 25-40 km from Filtu, namely Hayadimtu in the north-west, Bifatu PA in the south-east, Melkalibe PA in the north-east and Bakaka PA was conveniently included from the nearby Filtu town due to security reason.

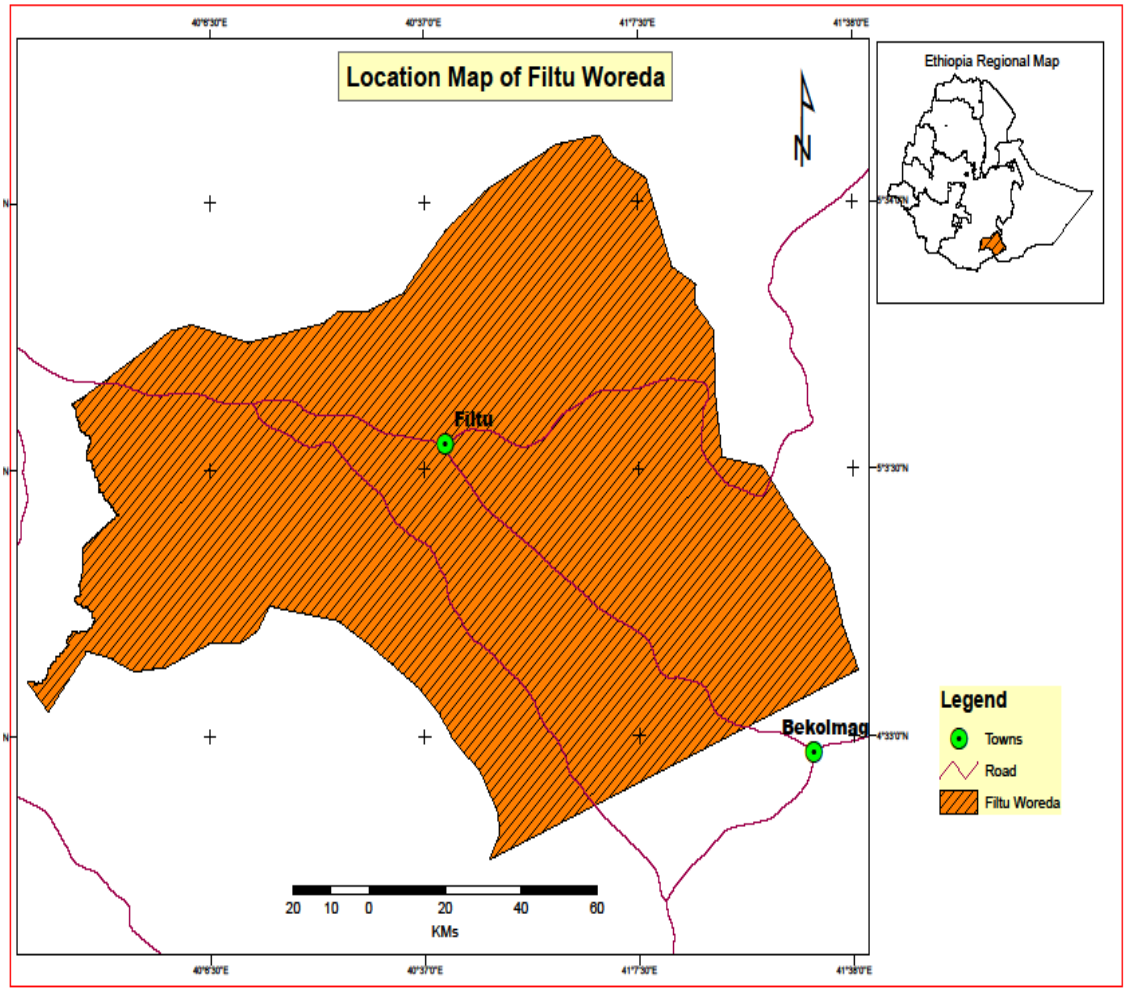

Figure 10 1 Map of study area

.Sample Size

The sample size for tuberculin testing was calculated using cluster sampling formula provided by (Bennett et al., 1991). Herds were considered as clusters. We assumed an intraclass correlation coefficient (rho) of 0.2, an expected prevalence of 3% and a standard error of 1.5%. The total sample size calculated was 1440 animals in 96 herds for all livestock species. The calculated sample size per livestock species was 480 in 32 herds.

In all four PAs, a list of households interested to participate in the study was established during general PA meetings and used as a sampling frame. Eight households were randomly selected from the list for each PA (using random number technique).

For each livestock species, 15 animals with age of greater or equal to one year were selected per herd and unique identification numbers were given for each tested animal

together with sex, age and body condition score (BCS). The animals were categorized into the following age groups: young (1-2.5 years), adult (2.6-6 years) and old (>6 years). BCS was assessed using a modified guideline described by Msangi et al. (1999) and cattle were classified as emaciated (score 1), thin (score 2), normal (score 3), musculous (score 4) and fat (score 5). For camels, BCS was based on hump scoring (amount of fat in the hump) which ranged from 1 - 5 http://www.camelsaust.com.au/livebodycond.htm. Goats were categorized as emaciated (1), normal (2) and well conditioned (3).

Tuberculin skin testing

The comparative intradermal tuberculin test (CIDT) was performed using both bovine and avian PPD obtained from the Veterinary Laboratories Agency, Addlestone, Surrey, United Kingdom**.**

Two injection sites were taken in the middle third of the side of the neck, one above the other, separated at least 12 cm for cattle and camels, while injection sites were taken on both sides of the neck in goats. The hair was shaved around the sites to a radius of about 2 centimeters. Skin fold at both sites were measured with a caliper and the measurements recorded. An aliquot of tuberculin containing 2,500 IU/0.1 ml bovine PPD was injected into the skin intradermally at the lower injection site and similarly tuberculin containing 2,500 IU/0.1 ml avian PPD injected at the upper site for cattle and camels, and for goats avian PPD on the right and bovine PPD on left side of the neck. After 72 hours, the thickness of the same skin fold at both sites was measured and recorded.

Bovine and avian positive reactors were obtained using the formula: $[(Bov_{72} - Bov_0)-(AV_{72}-Av_0)]$ and $[AV_{72}-Av_0)-(Bov_{72} - Bov_0)]$, respectively. Bov_0 and Av_0 indicated skin thickness before injecting bovine and avian tuberculin and Aov_{72} and Bov_{72} were the corresponding skin fold thickness 72 hours post-injection. The tuberculin test results were interpreted based on OIE recommended cut-off >4 mm. Increase in skin fold thickness >4 mm was regarded as positive reactor,1 to 4 mm doubtful reactor, negative if the increase in skin thickness at the bovine site of injection was less than the increase in the skin fold thickness at the avian site of injection.. Increase in skin fold thickness >1 mm with visible reaction at avian site than at the bovine site was considered as positive for *Mycobacterium avium spp*.

Questionnaire survey

To assess possible risk factors associated with husbandry practices and production system for tuberculin positivity and exposure to BTB infection, all herd owners of tuberculin-tested animals were interviewed using pre-tested structured questionnaires.

Data entry and analysis

The data was double entered in Microsoft Access 2002 (Microsoft Corp. Redmond, USA) and validated with EpiInfo version 3.3.2 before being imported to Stata 10/SE (Stata Corp., College Station, TX) for analysis.

The outcome of all statistical analyses was individual animal species and herd level binary outcomes. A herd was considered positive if it had at least one tuberculin reactor. Prevalence was calculated using xtgee model for each species.

Result

Individual animal prevalence

A total of 1418 animals from 94 randomly selected herds with the 34 goat herds, 32 camel herds and 28 cattle herds in Hayadimtu, Bifatu, Melkalibe and Bakaka PA's were tuberculin tested. A total of 421 cattle, 479 camels and 518 goats were tested. The individual animal prevalence were 2.0% (95% CI=0.5-8.4), 0.4% (95% CI=0.1-3%) and 0.2% (95% CI=0.03-1.3%) in cattle, camels and goats, respectively (Table 10.1). There was no significant difference in tuberculin positivity between animal species.

Prevalence of avian PPD reactors in cattle, camels and goats were 0.7% (95% CI 0.2-2.0%), 10.4 %(95% CI 7.0-14.0 %) and 1.9 (95% CI 0.9-4.0%), respectively. Camels had an OR of 16.5 (95% CI =5.0-55.0) for avian PPD positivity when compared to cattle.

Table 10 1 Prevalence of Bovine and avian PPD reactor animals, in cattle, camels and goats in study area

	Bovine PPD		Avian PPD		Univariate analysis	
Animal species	No test negative	No test positive (%), 95% CI	No test negative	No test positive (%), 95% CI	Bovine PPD OR (95% CI)	Avian PPD OR (95% CI)
Cattle	411	10 (2.0%), 0.5.0-8.4	317	3 (0.7), 0.2-2.0	1	1
Camel	477	2 (0.4), 0.1-3.0)	429	50 (10.4), 7-14.0	0.2 (0.01-4)	16.5(5.0-55.0)
Goats	517	1 (0.2), 0.03-1.3)	508	10 (1.9), 0.9-4.0	0.2 (0.01-3.0)	2.7(0.7-10.0)

Herd prevalence

Prevalence of bovine PPD reactor herds in cattle, camels and goats were 14.3% (95% CI 0.5-28.0%), 3.1% (95% CI -3.2-9.5%) and 2.9% (95% CI -3.0-9.0%), respectively, with no significant differences (Fisher's exact test) among herds of different animal species in BTB positivity (Table 10.2). No significant association was found between reactor herds and various risk factors.

Table 10 2 Herd prevalence among three livestock species, in study area

Animal species	Number of herd tested	Positive herd (%)	95 % CI
Cattle	28	4 (14.3)	0.5-28
camel	32	1 (3.1)	-3.2-9.5
Goats	34	1 (2.9)	-3.0-9.0

Discussion

The low BTB prevalences (<1.0% for camels and goats, and 2.0% for cattle) in our study was comparable with the reports from different regions of Tanzania 0.9% (Cleaveland et al., 2007), 0.7% (Weinhäupl et al., 2000), 1.3% (Shirima et al., 2003), 0.2% (Jiwa et al., 1997), Uganda 1.3% (Inangolet et al., 2008), 1.4% (Oloya et al., 2006), Ethiopia 0.9% (Tschopp et al., 2010). However, various other results were reported from Pakistan 2.4% in goats (Javed et al., 2010), Eritrea 14.5 % in cattle (Omer et al., 2001), Zambia 6.8% in cattle (Munyeme et al., 2009), Tanzania 13.2% in cattle (Kazwala et al., 2001) and from cattle in different regions of Ethiopia 46.8%, 19%, 11%, 9.7% and 11.6%, (Ameni et al., 2003; Shitaye et al., 2006; Ameni & Erkihun, 2007; Fetene & Kebede, 2009; Regassa et al., 2009), respectively. The inter-study variation may be due to differences in management practices, production system, types of animal species and breeds, or differences in

ecological zones. A higher BTB prevalence rate was reported in neighboring Oromia pastoralist in cattle 4.4% (Gumi et al., 2011). The pastoralist and agro-pastoralist production systems in neighboring Oromia pastoralist may explain this difference between the communities.

The prevalence of 10.4% (95% CI=7.0-14.0 %) camels to avian PPD in the present study is in line with a report of 10.0 % (Shirima et al., 2003) and 11.0% (Fetene & Kebede, 2009) in cattle from Zambia and Ethiopia, respectively. However, in contrast to our study, these studies also cattle and goats showed relatively high proportions of avian PPD reactors. The observed differences in prevalence of avian PPD among three livestock species that are kept and pastured together might be due to different susceptibility to non-BTB mycobacteria.

In our observations the herd prevalence of BTB are much lower compared to other authors (Kazwala et al., 2001; Omer et al., 2001; Ameni et al., 2003; Oloya et al., 2006; Shitaye et al., 2006; Oloya et al., 2007; Munyeme et al., 2008; Fetene & Kebede, 2009; Regassa et al., 2009) in the cattle herds in Ethiopia and the different countries of the region. This could be due to difference in agro-ecological zones and production systems.

Risk factors such as herd size, herd keeping with other livestock species, contact with other herds and annual migration dynamics, recent introduction of new animals to herd and other risk factors could not be associated with herd positivity to BTB. The high degree of similarities in a livestock management in pastoralist communities in study area may mask the effect of risk factors related to husbandry practices.

In the present study, prevalence of BTB was low in Somali pastoral livestock. The high proportion of camel reactors to avian PPD deserves further investigation of the responsible mycobacterial agent and a possible impact on livestock, in this case camel, productivity.

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Reference

Addo, K., Owusu-Darko, K., Yeboah-Manu, D., Caulley, P., Minamikawa, M., Bonsu, F., Leinhardt, C., Akpedonu, P., Ofori-Adjei, D.,2007. Mycobacterial Species Causing Pulmonary Tuberculosis at the Korle Bu Teaching Hospital, Accra, Ghana. Ghana Medical Journal 41(2), 52-57.

Ameni, G., Bonnet, P., Tibbo, M., 2003. A Cross-sectional Study of Bovine Tuberculosis in Selected Dairy Farms in Ethiopia. The International Journal of Applied Research in Veterinary Medicine 1(4), 85-97.

Ameni, G., Erkihun, A., 2007. Bovine tuberculosis on small-scale dairy farms in Adama Town, central Ethiopia, and farmer awareness of the disease. Revue Scientifique Et Technique (International Office of Epizootics) 26(3), 711-9.

Ayele, W.Y., Neill, S.D., Zinsstag, J., Weiss, M.G., Pavlik, I., 2004. Bovine tuberculosis: an old disease but a new threat to Africa. The International Journal of Tuberculosis and Lung Disease: The Official Journal of the International Union Against Tuberculosis and Lung Disease 8(8), 924-37.

Bennett, S., Woods, T., Liyanage, W.M., Smith, D.L.,1991. A simplified general method for cluster-sample surveys of health in developing countries. World Health Statistics Quarterly. Rapport Trimestriel De Statistiques Sanitaires Mondiales 44(3), 98-106.

Berg, S., Firdessa, R., Habtamu, M., Gadisa, E., Mengistu, A., Yamuah, L., Ameni, G., Vordermeier, M., Robertson, B.D., Smith, N.H., Engers, H., Young, D., Hewinson, R.G., Aseffa, A., Gordon, S.V., 2009. The Burden of Mycobacterial Disease in Ethiopian Cattle: Implications for Public Health. PLoS ONE 4(4), 5068.

Biet, F., Boschiroli, M.L., Thorel, M.F., Guilloteau, L.A., 2005. Zoonotic aspects of Mycobacterium bovis and Mycobacterium avium-intracellulare complex (MAC). Veterinary Research 36(3), 411-36.

Camels Australia Export - Central Australia - Northern Territory - Australia [Internet]. Available from: <http://www.camelsaust.com.au/livebodycond.htm> [Accessed 9 June 2010].

Cleaveland, S., Shaw, D.J., Mfinanga, S.G., Shirima, G., Kazwala, R.R., Eblate, E., Sharp, M., 2007. Mycobacterium bovis in rural Tanzania: Risk factors for infection in

human and cattle populations. Tuberculosis 87(1), 30-43.

Cosivi, O., Grange, J.M., Daborn, C.J., Raviglione, M.C., Fujikura, T., Cousins, D., Robinson, R.A., Huchzermeyer, H.F., de Kantor, I., Meslin, F.X., 1998. Zoonotic tuberculosis due to Mycobacterium bovis in developing countries. Emerging Infectious Diseases 4(1),.59-70.

Demelash, B., Inangolet, F., Oloya, J., Asseged, B., Badaso, M., Yilkal, A., Skjerve, E., 2009. Prevalence of Bovine tuberculosis in Ethiopian slaughter cattle based on post-mortem examination. Tropical Animal Health and Production 41(5), 755-765.

Fetene, T., Kebede, N., 2009. Bovine tuberculosis of cattle in three districts of northwestern Ethiopia. Tropical Animal Health and Production 41(2), 273-277.

Godreuil, S., Renaud, F., Choisy, M., Depina, J.J., Garnotel, E., Morillon, M., Van de Perre, P., Bañuls, A.L., 2010. Highly structured genetic diversity of the Mycobacterium tuberculosis population in Djibouti. Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases 16(7), 1023-1026.

Gumi, B., Schelling, E., Firdessa, R., Aseffa, A., Tschopp, R., Yamuah, L., Young, D. Zinsstag, J., 2011. Prevalence of bovine tuberculosis in pastoral cattle herds in the Oromia region, southern Ethiopia. Tropical Animal Health and Production, Available from: <http://www.ncbi.nlm.nih.gov/pubmed/21499975> [Accessed 19 April 2011].

Hiko, A., Agga, G.E.,2011. First-time detection of mycobacterium species from goats in Ethiopia. Tropical Animal Health and Production 43(1), 133-139.

Idigbe, E.O., Anyiwo, C.E., Onwujekwe, D.I., 1986. Human pulmonary infections with bovine and atypical mycobacteria in Lagos, Nigeria. The Journal of Tropical Medicine and Hygiene 89 (3), 143-148.

Inangolet, F.O., Demelash, B., Oloya, J., Opuda-Asibo, J., Skjerve, E., 2008. A cross-sectional study of bovine tuberculosis in the transhumant and agro-pastoral cattle herds in the border areas of Katakwi and Moroto districts, Uganda. Tropical Animal Health and Production 40(7), 501-8.

Javed, M.T., Munir, A., Shahid, M., Severi, G., Irfan, M., Aranaz, A., Cagiola, M., 2010. Percentage of reactor animals to single comparative cervical intradermal tuberculin (SCCIT) in small ruminants in Punjab Pakistan. Acta Tropica 113(1), 88-91.

Jiwa, S., Kazwala, R., Aboud, A., Kalaye, W., 1997. Bovine tuberculosis in the Lake Victoria zone of Tanzania and its possible consequences for human health in the HIV/AIDS era. Veterinary Research Communications 21(8),533-539.

Kazwala, R., Kambarage, D., Daborn, C., Nyange, J., Jiwa, S., Sharp, J., 2001. Risk Factors Associated with the Occurrence of Bovine Tuberculosis in Cattle in the Southern Highlands of Tanzania. Veterinary Research Communications 25(8), 609-614.

Mamo, G., Kassaye, A., Sanni, M., Ameni, G., 2009. A cross sectional study of camel tuberculosis in Ethiopia. Bulletin of Animal Health and Production in Africa, 57(1).

Mamo, G., Bayleyegn, G., Sisay Tessema, T., Legesse, M., Medhin, G., Bjune, G., Abebe, F., Ameni, G., 2011. Pathology of camel tuberculosis and molecular characterization of its causative agents in pastoral regions of ethiopia. PloS One 6(1), 15862.

Michel, A.L., Müller, B., van Helden, P.D., 2010. Mycobacterium bovis at the animal-human interface: a problem, or not? Veterinary Microbiology 140(3-4), 371-381.

Msangi, B.S.J., Bryant, M., Kavana, P., Msanga, Y., Kizima, J.,1999. Body measurements as a management tool for crossbred dairy cattle at a farm Smallholder condition. In: Proceedings of the meeting in The Tanzanian Society of Animal Production. Tanzania.

Munyeme, M., Muma, J.B., Skjerve, E., Nambota, A.M., Phiri, I.G.K., Samui, K.L., Dorny, P., Tryland, M., 2008. Risk factors associated with bovine tuberculosis in traditional cattle of the livestock/wildlife interface areas in the Kafue basin of Zambia. Preventive Veterinary Medicine 85(3-4), 317-28.

Munyeme, M., Muma, J., Samui, K., Skjerve, E., Nambota, A., Phiri, I., Rigouts, L. Tryland, M., 2009. Prevalence of bovine tuberculosis and animal level risk factors for indigenous cattle under different grazing strategies in the livestock/wildlife interface areas of Zambia. Tropical Animal Health and Production 41(3), 345-352.

OIE, 2009. Office International des Epizooties Terrestrial manual: Chapter 2.4.7, World Health Organization for Animal Health, Paris.

Oloya, J., Opuda-Asibo, J., Kazwala, R., Demelash, A., Skjerve, E., Lund, A., Johansen, T., Djonne, B., 2008. Mycobacteria causing human cervical lymphadenitis in pastoral

communities in the Karamoja region of Uganda. Epidemiology and Infection136(5), 636-643.

Oloya, J., Muma, J.B., Opuda-Asibo, J., Djønne, B., Kazwala, R., Skjerve, E., 2007. Risk factors for herd-level bovine-tuberculosis seropositivity in transhumant cattle in Uganda. Preventive Veterinary Medicine 80 (4), 318-29.

Oloya, J., Opuda-Asibo, J., Djønne, B., Muma, J., Matope, G., Kazwala, R., Skjerve, E. 2006. Responses to tuberculin among Zebu cattle in the transhumance regions of Karamoja and Nakasongola district of Uganda. Tropical Animal Health and Production 38 (4), 275-283.

Omer, M.K., Skjerve, E., Woldehiwet, Z., Holstad, G., 2001. A cross-sectional study of bovine tuberculosis in dairy farms in Asmara, Eritrea. Tropical Animal Health and Production 33(4), 295-303.

Regassa, A., Tassew, A., Amenu, K., Megersa, B., Abunna, F., Mekibib, B., Marcotty, T., Ameni, G., 2010. Erratum to: A cross-sectional study on bovine tuberculosis in Hawassa town and its surroundings, Southern Ethiopia. Tropical Animal Health and Production 42(4), 1039.

Schiller, I., Vordermeier, H.M., Waters, W.R., Whelan, A.O., Coad, M., Gormley, E., Buddle, B.M., Palmer, M., Thacker, T., McNair, J., Welsh, M., Hewinson, R.G. Oesch, B.,2010. Bovine tuberculosis: Effect of the tuberculin skin test on in vitro interferon gamma responses. Veterinary Immunology and Immunopathology136(1-2) , 1-11.

Shirima, G.M., Kazwala, R.R.; Kambarage, D.M., 2003. Prevalence of bovine tuberculosis in cattle in different farming systems in the eastern zone of Tanzania. Preventive Veterinary Medicine 57 (3),167-72.

Shitaye, J., Getahun, B., Alemayehu, T., Skoric, M., Treml, F., Fictum, P., Vrbas, V. Pavlik, I., 2006.A prevalence study of bovine tuberculosis by using
abattoir meat inspection and tuberculin skin testing
data, histopathological and IS6110 PCR examination
of tissues with tuberculous lesions in cattle in Ethiopia. Veterinarni Medicina 51 (11), 512–522.

Shitaye, J., Tsegaye, W. Pavlik, I., 2007. Bovine tuberculosis infection in animal and

human
populations in Ethiopia: a review. Veterinarni Medicina 52 (8), 317–332.

Tschopp, R., Schelling, E., Hattendorf, J., Young, D., Aseffa, A.,Zinsstag, J., 2010. Repeated cross-sectional skin testing for bovine tuberculosis in cattle kept in a traditional husbandry system in Ethiopia. The Veterinary Record 167(7), 250-256.

Weinhäupl, I., Schöpf, K.C., Khaschabi, D., Kapaga, A.M., Msami, H.M., 2000. Investigations on the prevalence of bovine tuberculosis and brucellosis in dairy cattle in Dar es Salaam region and in zebu cattle in Lugoba area, Tanzania. Tropical Animal Health and Production 32 (3), 147-54.

Zinsstag, J., Schelling, E., Roth, F., KAZWALA, R., 2006. Economics of Bovine Tuberculosis. In: Mycobacterium bovis infection in animals and humans. Boston, Wiley-Blackwell, p.360.

11. Zoonotic transmission of tuberculosis between pastoralists and their livestock in South-East Ethiopia

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Abstract

Setting: Despite huge global efforts in tuberculosis (TB) control, pastoral areas remain under-investigated.

Objective: To investigate the presence of zoonotic transmission of tuberculosis in Ethiopian pastoralists of Oromia and Somali Regional States.

Design: From March 2008 to February 2010 sputum and fine-needle aspirate (FNA) specimens were collected from 260 patients with suspected pulmonary TB and from 32 cases with suspected TB lymphadenitis. In parallel, 207 suspected tuberculous lesions were collected from cattle, camels and goats at abattoirs. All specimens were processed and cultured for mycobacteria; samples with acid-fast stained bacilli (AFB) were further characterized by molecular methods including genus and deletion typing as well as spoligotyping. Non-tuberculous mycobacteria (NTM) were sequenced at the 16S rDNA locus.

Results: Culturing of AFB from human sputum and FNA samples gave a yield of 174 (67%) and nine (28%) isolates, respectively. Molecular typing was performed on 173 of these isolates and 160 were confirmed as *Mycobacterium tuberculosis*, three as *Mycobacterium bovis*, and the remaining 10 were typed as NTMs. Similarly, 48 AFB isolates (23%) yielded from tuberculous lesions of livestock, of which 39 were molecular typed, including 24 *M. bovis* and four NTMs from cattle, one *M. tuberculosis* and one NTM from camels, and nine NTMs from goats.

Conclusion: Isolation of *M. bovis* from humans and *M. tuberculosis* from livestock suggests transmission between livestock and humans in the pastoral areas of South-East Ethiopia.

Introduction

Ethiopia ranks seventh among the world's 22 countries with high tuberculosis disease burden and had an estimated incidence rate of 379 cases per 100,000 people per year (WHO, 2008). *M. tuberculosis* is the most common cause of human TB, but an unknown proportion of cases are due to *M. bovis*. TB caused by *M. bovis* (bovine tuberculosis; bTB) is clinically indistinguishable from TB caused by *M. tuberculosis* and can only be differentiated by laboratory methods (Cosivi et al., 1998). Specific data on zoonotic bTB transmission is very scarce in the developing world because the diagnosis of TB most often relays on sputum microscopy only. However, fairly recent molecular methods like spoligotyping (Kamerbeek et al. 1997) and deletion typing (Brosch et al. 2002) allow for identification of *M. bovis*.

Although cattle are considered to be the main hosts of *M. bovis*, isolations have been made from many other livestock and wildlife species and transmission to humans constitutes a public health problem (Ayele et al., 2004; OIE, 2009). In many developing countries, bTB remains endemic causing significant economic losses (Zinsstag et al., 2006). In animals, bTB has been reported from 33 of 43 African countries (Ayele et al., 2004). Human cases of bTB have been described in some Sahelian countries like Ghana, Niger, Uganda and Tanzania (Idigbe et al., 1986; Addo et al., 2007; Oloya et al., 2008) and in immigrants from Chad (Godreuil et al., 2010).

The proportion of bTB in human TB is estimated to be less than 5% worldwide (Cosivi et al., 1998; Michel et al., 2010). But this figure is based on estimates and we lack empirical representative data on the proportion of human *M. bovis* among all TB patients at national level. This information would be important for the estimation of the societal cost of bTB.

Routes of transmission to people are likely to be through consumption of untreated milk and meat products from infected animals, but also via aerosol in the proximity to livestock. These possible risk factors are of particular concern for many developing countries where pasteurization is limited and where people are living close to their animals.

In Ethiopia, several prevalence studies have been performed recently that show that bTB is endemic in cattle; however, prevalences vary depending on the geographical areas, breeds and husbandry practices. Abattoir and dairy farm studies from central Ethiopia have reported prevalence between 3.5%-13.5% and locally in peri-urban Addis Ababa up to 50%

(Ameni et al., 2007; Shitaye et al., 2007; Berg et al., 2009; Demelash et al., 2009; Regassa et al., 2010). In contrast, lower prevalence of 0.9% was reported in traditionally-kept zebu cattle (Tschopp et al., 2010a). Other livestock than cattle have also been investigated. Based on gross pathology, prevalences of 5-10% were reported in camels slaughtered at Dire Dawa abattoir in eastern Ethiopia and in Addis Ababa abattoir (Mamo et al., 2009; Mamo et al., 2011). Hiko & Agga, (2011) reported a 4.2% prevalence of bTB in goats slaughtered at the Mojo export abattoir in central Ethiopia. The observed variability of bTB disease frequency in Ethiopia might well be influenced by different livestock production systems (rural/pastoral/peri-urban) and different geographic and climatic contexts. Transmission of bTB seems to be higher in intensive peri-urban settings when compared to extensive rural and pastoral areas. Hence, a detailed understanding of bTB transmission requires field studies in a given social and ecological context.

Most of these studies focused on prevalence in cattle in the central highlands of Ethiopia. However, little data on human and animal TB is available from the major pastoralist areas in South-East Ethiopia, particularly from the Somali Region, which are often difficult to access also due to insecurity. Few studies have been conducted in southern Ethiopia. The abattoir bTB prevalence of Borana pastoralist cattle was 4% (Demelash et al., 2009), individual comparative intradermal tuberculin test prevalences were 0.8% in cattle of Hamer pastoralists in South Omo (Tschopp et al., 2010b) and 4.4% among Guji-Boran pastoralist cattle (Gumi et al., 2011). It appears that the prevalence of bTB in pastoral areas of Southern Ethiopia is relatively low. However, since pastoralists live in close proximity with their animals, animal-to-human transmission of bTB might still be significant. The potential of transmission of zoonotic TB in South-East Ethiopia was unknown. The objectives of this study were firstly to assess the presence of *M. bovis* among human TB patients and to describe mycobacterial strains circulating in South-East Ethiopian pastoralists and their livestock using a “One health” approach, studying human and livestock hosts simultaneously (Zinsstag et al., 2009). Secondly, data from this study should then be compared with the overall epidemiological situation in Ethiopia.

Materials and methods

Study Area

The study was conducted from March 2008 to February 2010 in southeast Ethiopia in the Guji (Negelle) and Liben (Filtu) zones of Oromia and Somali Regional States (Figure 8.1). The lowland of the Guji zone is inhabited by pastoral and agro-pastoral communities whose livelihood is based on livestock production. The Liben (Filtu) zone of Somali region is arid lowland inhabited by pastoral communities. The study area has the highest livestock population density in the country and is a major source of livestock for the domestic and export markets. Cross-border movement of pastoral communities and their livestock to neighboring countries (Kenya and Somalia) is common.

Sample collection

Ethical clearance for the study was obtained from the Ethics committee of Basel (Ref. 147/08, AHRI/ALERT; Ref. P010/08) and the Ethiopian National Ethical Review Committees (Ref. RDHE/65-86/2009). After informed consent was obtained from participants, sputum samples from suspected pulmonary TB patients and fine needle aspirates (FNA) samples from suspected TB lymphadenitis patients were collected by trained laboratory technicians or physicians. FNA specimens were collected and stored in cryo-tubes with phosphate buffer saline (PBS) pH 7.2, and sputum specimens were collected in sterile containers. Suspected tuberculous lesions were collected by trained meat inspectors from cattle carcasses at Negelle abattoir and from camels and goats slaughtered at Filtu slaughterhouse. Similarly, sampling of suspected tuberculous lesions was also performed on camels and goats that were traceable back to the pastoral areas in South-East Ethiopia at Mojo and Addis Ababa abattoirs. All animal specimens were preserved in PBS in 30ml plastic sterile universal containers. All human and animal specimens were stored at 4°C until transported on ice within five days to the Armauer Hansen Research Institute (AHRI) laboratory in Addis Ababa. In case of unavailable transportation means, samples were kept in the regions at -20 °C before transport and further processing at AHRI. A flow chart of the sample collection, processing and molecular typing is provided in Figure 8.2.

Culturing and molecular typing

All specimens were processed according to standard methods. Tuberculous-like animal lesions were dissected and manually homogenized, then decontaminated with 4% NaOH

for 15min and centrifuged at 3,000 rpm for 15min. The sediment was neutralized with 2N HCl using phenol red as an indicator and inoculated on three different media slants: Two Löwenstein–Jensen (LJ) media supplemented either with glycerol or pyruvate, and Middlebrook 7H11 media (Berg, et al., 2009). The slants were incubated at 37°C for eight weeks and examined daily for the first week and then weekly for the presence of mycobacterial colonies. Cultures were considered negative if no visible growth was detected after eight weeks of incubation. Microscopic examination of cultures using the Ziehl–Neelsen staining method was performed to detect presence of acid-fast positive bacilli (AFB) (Roberts et al. 1991). AFB positive cultures were prepared as 20% glycerol stocks and stored at –80°C as reference.

Heat-killed cells of each AFB isolate were prepared by mixing ~2 loopfuls of cells (≥ 20µl cell pellet) in 200µl dH_2O followed by incubation at 80°C for 1 hour. Heat-killed AFB samples were used as templates in multiplex polymerase chain reactions (PCR) for typing of *Mycobacterium* genus and Region of Difference (RD; deletion typing), according to protocols previously described (Berg, et al., 2009). Each isolate characterized as non-tuberculous mycobacteria (NTM) was sequenced at the 16S rDNA locus and the sequence was entered in the Basic Local Assignment Search Tool (BLAST) database at the National Center for Biotechnology Information (NCBI) and the Ribosomal Differentiation of Microorganisms (RIDOM) (http://rdna.ridom.de) database for further identification of species (Berg et al., 2009). DNA sequencing was performed at the Animal Health and Veterinary Laboratories Agency (AHVLA), United Kingdom, using an Applied Biosystems model 3730 automated capillary DNA sequencer. Isolates genetically identified by deletion typing as of the *M. tuberculosis* complex (MTC) were spoligotyped for further strain characterization as previously described (Kamerbeek et al., 1997). Spoligotyping data were compared with the Spoligo-International-Typing (SIT) database (http://www.pasteur-guadeloupe.fr:8081/SITVITDemo/ and http://www.cs.rpi.edu/~bennek/tbinsight/tblineage.html to match SIT numbers and lineage classifications. Isolates identified as *M. bovis* were compared with spoligotype patterns in the international *M. bovis* database (www.mbovis.org). Spoligotype patterns of all MTC isolates were analysed using spolTools (http://www.emi.unsw.edu.au/spolTools) (Tang et al., 2008).

Molecular typing methods (adapted from (Müller 2008))

Spacer oligonucleotide typing (Spoligotyping)

Spoligotyping makes use of the variability of the MTC chromosomal direct repeat (DR) locus for strain differentiation (Kamerbeek et al. 1997). The DR region is composed of multiple well conserved direct repeats of 37 bp which are separated by non-repetitive 34 to 41 bp spacer sequences. In the standard spoligotyping scheme, a PCR with primers complementary to the DR-sequence is used to amplify all spacer sequences of a given strain. One of the two primers is labelled with a biotin marker. The PCR products are denatured and hybridized to a standard set of 43 oligonucleotides covalently linked to a membrane. These oligonucleotides correspond to 37 spacers from *M. tuberculosis* H37Rv and 6 additional spacers from *M. bovis* BCG P3. If any of these spacers are also present in an investigated strain, they will be amplified during the PCR and hybridized to the spacers on the membrane. The successful hybridization can be visualized by incubation with Streptavidin peroxidase (which binds to the biotin molecule), subsequent addition of a chemiluminescent Streptavidin peroxidase substrate and exposure to a light sensitive film. The presence or absence of each individual spacer sequence will generate a spoligotype pattern for the specific strain that was typed.

Large sequence polymorphism (LSP) analysis

LSPs generally refer to large genomic deletions or insertions. Large genomic deletions (also called regions of difference, RD) are widely used for the phylogenetic analyses of MTC but are not appropriate for molecular epidemiological studies due to a low mutation rate (Gagneux and Small 2007). The genomic deletion RD9 discriminates *M. tuberculosis* from the other members of the MTC. If RD9 is intact, a strain is considered as *M. tuberculosis*. Similarly, *M. bovis* is deleted for RD4 while all other members of the MTC are not. Thereby, deletion typing of RD4 allows for identification of *M.bovis.*

Results

Sample collection and culturing yield

A total of 292 patients clinically diagnosed with either pulmonary TB or TB lymphadenitis were recruited in Negelle and Filtu hospitals (Table 11.1). Sputum of 260 TB cases was cultured with a culturing yield of 164 (67%) AFB positive isolates, while FNA samples were taken from 32 cases with TB lymphadenitis of which culturing yielded in nine (28%)

AFB positive isolates (Table 11.1). In parallel, 207 samples were collected from cattle, camels and goats with suspected TB lesions in Negelle, Filtu, Mojo, and Addis Ababa abattoirs. Culturing yielded in 48 (23%) isolates that were identified as AFB positive isolates (Table 11.2).

Table 11.1: Numbers of human specimen that were cultured and RD9 deletion typed from sputum and fine needle aspirates (FNA) from Negelle and Filtu Hospital.

Specimen	Negelle Hospital	Filtu Hospital	AFB positive	Deletion typing	*M. tuberculosis*	*M. bovis*	NTM[a]	Spol[b]
Sputum	192	68	174	164	154	3	7	156
FNA	14	18	9	9	6	0	3	5
Total	206	86	183	173	160	3	10	161

AFB positive = number of are acid-fast positive sputa and FNA samples, RD9 deletion = number of strains on which RD 9 was tested, *M. tuberculosis* = number of *M. tuberculosis* strains, *M. bovis* = number of *M. bovis* strains, [a] NTM = Typed as *Mycobacterium* species not from the *M. tuberculosis* complex, [b] Spol = isolates available for spoligotype analysis

Table 11.2 Numbers of abattoir specimen that were cultured and RD4 deletion typed from Negelle, Filtu, Addis Ababa and Mojo

Livestock species investigated (N)	Sites	Colleted and Processed specimens	AFB positive	Deletion typing	*M. tuberculosis*	*M. bovis*	NTM[a]	Spol[b]
Cattle (5250)	Negelle	50	36	28	0	24	4	24
Camels (694)	Filtu=181 Addis Ababa=513	81	3	3	1	0	1	1
Goats (1744)	Filtu=244 Mojo=1500	76	9	9	0	0	9	0
Total		207	48	40	1	24	14	25

AFB positive = number of are acid-fast positive sputa and FNA samples, RD4 deletion = number of strains on which RD 4 was tested, *M. tuberculosis* = number of *M. tuberculosis* strains, *M. bovis* = number of *M. bovis* strains, NTM[a] = Typed as *Mycobacterium* species not from the *M. tuberculosis* complex, Spol[b] = isolates available for spoligotype analysis, N=number of animals inspected

RD4 and RD9 deletion typing

To further characterise the AFB isolates, we used deletion typing to identify strains from the *M. tuberculosis* complex (MTC). Out of the 164 sputum isolates tested for RD9, 154 had intact RD9 locus and were subsequently classified as *M. tuberculosis*, while three isolates were RD9-deleted. The latter strains were also found to be deleted for RD4, a characteristic of *M. bovis*, and were declared as *M. bovis* strains. Assays of the remaining

seven isolates did not generate any PCR product and were classified as NTM. Among the nine FNA isolates, six were *M. tuberculosis* (RD9 intact), whereas three isolates were NTM (Table 11.1).

A total of 40 livestock isolates were RD4 deletion typed, of which 28, three, and nine isolates were from cattle, camels, and goats, respectively (Table 11.2). Out of 28 cattle isolates, 24 were *M. bovis* (RD4 deleted), while four were suggested as NTM. Among the three isolates from camels, one was characterized as *M. tuberculosis* from an animal with disseminated TB lesions (Figure 11.1), one was suggested as an NTM and one requires further characterization. None of the nine AFB isolates from goats were typed as of MTC.

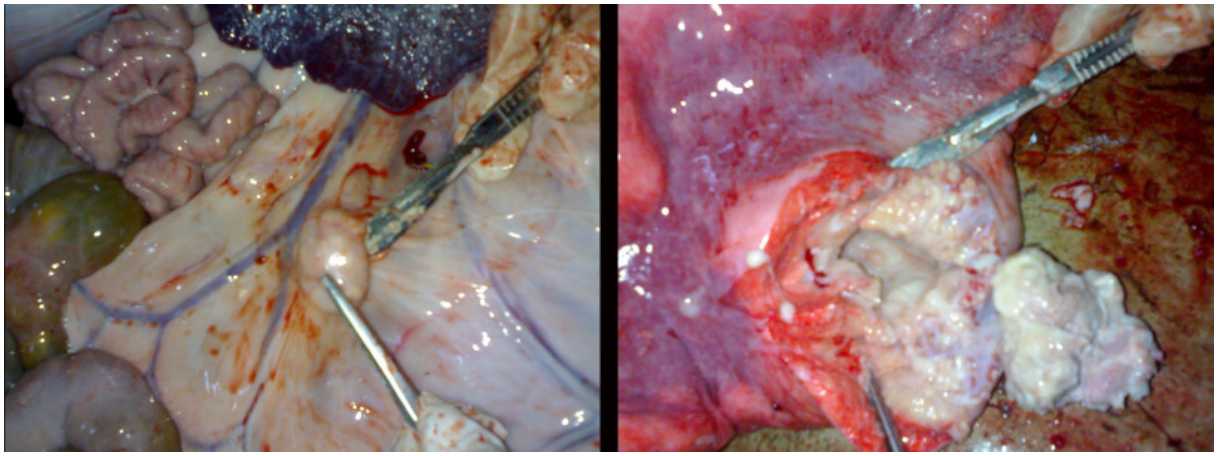

Figure 11.1 TB lesions from camel: enlarged mesenteric lymph node (left) and cross-section of TB lesion in the lung (right). Mycobacterium isolated from this lesion was characterized as *M. tuberculosis* (Photo: E. Meles)

Genus typing and 16S rDNA sequencing

Isolates that had failed to produce a PCR product in the deletion typing assays were tested by genus typing. In total, 24 isolates were NTM (Table 11.1 and 2) and six of these isolates were further identified by partial sequencing of the 16S rDNA gene. Three isolates were from goats were *M. terrae* complex strains, *M. arupense*, and *Corynebacterium pseudotuberculosis*, while two isolates from cattle were both *M. fortuitum*. One of the ten human NTM isolates was characterized as *M. flavescens* (Table 11.3).

Table 11.3: Identified non-complex mycobacteria (NTM) isolates from 16SrDNA locus sequencing results

.Bacterial species	Source
Mycobacterium terrae complex	1 goat
Mycobacterium arupense	1 goat
Corynebacterium pseudotuberculosis	1 goat
Mycobacterium fortuitum	2 cattle
Mycobacterium flavescens	1 human-sputum

Spoligotyping of human isolates

A total of 161 strains from sputum and FNA samples were spoligotyped. Five lineages could be recognized in this strain collection based on spoligotype features characteristic for each lineage (Brudey et al., 2006; Comas et al., 2009). The Euro-American (E-A) and the Central-Asian (CAS) lineages were dominating with 73.3% and 17.4%, respectively, while 6.8% of the strains belonged to the East-African-Indian (EAI) lineage. One isolate (0.6%) had the Beijing spoligotype pattern and the three *M. bovis* strains (1.9%) were also recognized by their characteristic spoligotype feature with missing spacers 3, 9, 16 and 39-43 (Table 11. 4). Strains characterized as *M. tuberculosis* by the deletion typing were represented by 48 different spoligotype patterns, of which 15 had not yet been registered in SpolDB4, the international spoligotyping database (Brudey et al., 2006). Among the unregistered patterns, eleven strains belonged to the E-A lineage, one to the CAS and one to the EAI lineage. Nineteen strains were clustered with an average cluster size of 3 and a clustering ratio of 0.32, while 31 strains had unique patterns. The largest cluster belonged to the spoligotype SIT 149 and accounted for 24% of the 161 isolates. Strains of this type lack spacers 10-19 and 33-36, and constitute a sublineage named T3-ETH (Brudey et al., 2006). Two other more common patterns were SIT37 of the E-A lineage and SIT25 of the CAS lineage. The three *M. bovis* strain isolated from sputum were of spoligotypes SB0133 and SB0303 in the Mbovis.org database.

Spoligotyping of animal isolates

A total of 25 MTC strains from livestock were characterized by spoligotyping. The 24 strains of *M. bovis* isolated from cattle were represented by six different spoligotype patterns, two clusters and four unique patterns. The average cluster size was 3.6 with a clustering ratio of 0.3.

The vast majority of livestock *M. bovis* isolates were of spoligotype SB0133, while the remaining strains showed a slightly diverting spoligotype patterns SB0933, SB1942, and SB1983 (Table 11.4). The latter two types were new to the database Mbovis.org. All *M. bovis* strains lacked spacers 3 to 7, 9, 16, and 39-43. The single *M. tuberculosis* strain isolated from camel had spoligotype SIT 149 of the E-A lineage; in this study most commonly isolated *M. tuberculosis* type.

Table 11. 4. Spoligotypes of *M. tuberculosis* Complex Strains Isolated from Humans and Livestock in South-Eastern Ethiopia

A) *M. tuberculosis* spoligotype patterns																																															Human		Livestock		
SIT No	Spoligotypes; showing presence (box) or absence (blank) of spacer 1 to 43																																										Lineage*	Sub-lineage*	SP	FN	Ct	Cm	Total
SIT 53	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	E-A	T1	8				8
SIT 37	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	E-A	T3	10				10
SIT 3137	■	■	■	■	■	■	■	■		■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	E-A		2				2
New							■	■		■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	E-A		1				1
SIT 73	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■					■	■	■		■	■	■	E-A	T3 variant	1				1
New	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■				■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	E-A		1				1
New	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■								■	■	■					■	■	■	■	■	■	■	E-A		1				1
SIT 54	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	E-A		2				2
New	■	■	■	■		■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	E-A		5				5
SIT 52	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■					■	■	■		■	■	■	E-A	T2	8	1			9
New	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■		■		■					■	■	■	■	■	■	■	E-A		1				1
SIT 134	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■					■	■			■	■	■	E-A	H3 variant	5				5
SIT 1457	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■				■					■	■	■	■	■	■	■	E-A		1				1
New	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			■	■	■	■				■					■	■	■	■	■	■	■	E-A		1				1
SIT 817	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■				■					■	■	■		■	■	■	E-A		1				1
SIT 42	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	E-A		1				1
New	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	■					■	■	■				■	E-A		1				1
New		■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	E-A		4				4
SIT 59	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■					■	■					■	■					■	■	■	■	■	■	■	E-A	LAM11-ZWE	1				1
SIT 47	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							■					■	■	■	■	■	■	■	E-A	H1	1				1
SIT 62	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							■					■	■	■		■	■	■	E-A	H1 variant	1				1
New	■	■		■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■				■					■	■	■		■	■	■	E-A		1				1
SIT 149	■	■	■	■	■	■	■	■	■											■	■	■	■	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	E-A	T3-ETH	37	1		1	39
New	■	■	■	■	■	■	■	■	■											■	■	■	■	■	■	■	■	■		■	■	■					■	■	■	■	■	■	■	E-A		1				1
SIT 3141			■	■	■	■	■	■	■											■	■	■	■	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	E-A		2				2
SIT 41	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■						■			■	■	■	■	■					■	■	■	■	■	■	■	E-A	LAM07-TUR	6				6
New	■																	■	■	■	■		■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	E-A		2				2
SIT 4																									■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	E-A	LAM3/S	8	1			9
New	■	■	■					■	■		■	■	■	■	■	■	■	■	■						■	■	■	■	■	■	■	■				■	■	■	■	■	■	■	■	CAS		1				1
SIT 1675	■	■	■					■	■		■	■	■	■		■	■	■	■																■	■	■	■	■	■	■	■	CAS		1				1
SIT 21	■	■	■					■	■		■	■	■	■	■	■	■	■	■																■	■	■	■	■	■	■	■	CAS	CAS1-Kili	7				7
SIT 25	■	■	■					■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■													■	■			■	■	■	■	■	CAS	CAS1-variant	10				10
SIT 1198	■	■	■					■	■	■	■	■		■	■	■	■	■	■	■	■	■													■	■			■	■	■	■	■	CAS		1				1
SIT 2359	■	■	■					■	■	■	■		■	■	■	■	■	■	■	■	■	■													■	■			■	■	■	■	■	CAS			1			1
SIT 247	■	■	■					■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■													■	■	■			■	■	■	■	CAS		2				2
SIT 142	■	■	■					■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■														■	■	■	■	■	■	■	■	■	CAS		1				1
SIT 26	■	■	■					■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■													■	■	■	■	■	■	■	■	■	CAS	CAS1-Delhi	4				4
New	■	■	■					■	■	■					■	■	■	■	■	■	■	■													■	■	■	■	■	■	■	■	■	CAS		1				1
SIT 1																																			■	■	■	■	■	■	■	■	■	Beijing	Beijing	1				1
SIT 10	■			■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■		■	■	■	■	■	■	■					■		■	■	■	■	■	■	■	■	■	EAI		1				1
SIT 24	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■					■		■	■	■	■	■	■	■	■	■	EAI		1				1
SIT 591	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■					■		■	■	■	■	■	■	■	■	■	EAI	EAI6-BGD1	1				1
SIT 43	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			■	■	■	■					■		■	■	■	■	■	■	■	■	■	EAI		1				1
New	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			■	■	■	■					■		■	■	■	■	■	■	■	■	■	EAI			1			1
SIT 48	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■					■		■	■	■	■	■		■	■	■	EAI	EAI1-SOM	2				2
SIT 6				■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■					■		■	■	■	■	■		■	■	■	EAI		1				1
New	■	■	■	■	■	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■	■						■					■		■		■	■	■	■	■	■	■	EAI		2				2
SIT 924	■	■	■	■	■	■	■	■	■	■	■														■	■	■	■					■		■	■		■	■	■	■	■	■	EAI		1				1
B) *M. bovis* spoligotype patterns																																																		
SB0133	■	■						■		■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							African 2		1		17		18
SB0303	■	■						■		■	■	■		■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							African 2		2				2
New	■	■						■			■	■	■				■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■							African 2				1		1
New	■	■						■			■	■	■				■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■								African 2				1		1
SB0933	■	■						■		■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■								African 2				3		3
SB1942	■	■						■		■	■	■	■	■	■		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			■	■							African 2				1		1
SB1983	■	■						■		■	■	■	■	■	■		■	■	■	■	■	■	■	■	■								■	■	■	■	■	■	■							African 2				1		1
																																													Total number of isol	156	5	24	1	186

* Lineages/sub-lineages as defined by Brudey et al. 2006 and Berg et al., 2011.
SIT No = Spoligo-International-Typing as described in SpolDB4 (Brudey et al., 2006); SB No = www.mbovis.org
SP=sputum; FN= FNA; Ct= cattle; Cm= camel; E-A= Euro-American; CAS= Central-Asian; EAI=East-African-Indian

Discussion

Human isolates

This study suggests strongly that transmission of the causative agents of TB occur between humans and livestock in the pastoralist settings of Ethiopia. Indeed, our most recent typing data shows the exact same spoligotype and 24-loci MIRU-VNTR type (data not shown) for human sputum and a cattle *M. bovis* isolate from the study area. However, despite close contact between humans and livestock, including consumption of raw milk and meat by pastoral communities, the incidence rate and subsequent prevalence of *M. bovis* in human TB patients was lower than expected. Higher prevalences of *M. bovis* in human TB patients have been reported in Uganda (7%) (Oloya et al., 2008), Ghana (3%) (Addo et al., 2007), and in Nigeria (4% and 15%) (Idigbe et al., 1986; Mawak et al., 2006). This variation may be due to differences in transmission pathways or in sampling and diagnostic techniques, for example, Oloya et al (2008) sampled from lymph node biopsy of cervical TB lymphadenitis instead of FNA. The former produces higher culture yields. However, a recent study from central Ethiopia identified no *M. bovis* in patients with TB lymphadenitis when sampling from lymph node biopsies were performed (Beyene et al., 2009).

Even if the true prevalence of TB due to *M. bovis* among the pastoralists in Ethiopia is not yet well understood, this study shows that a few percents of the pastoralists suffering from TB may in fact have bTB (in our study 2%). Therefore, in a country with a high TB burden as is the case with Ethiopia, the number of patients that may require treatment for bTB may become critical. It is interesting to notice that the three patients identified with bTB had pulmonary disease and it raises the question if transmission by aerosol rather than by ingestion of contaminated food products were the causes of infection. The observed low numbers of recruited TB lymphadenitis cases in this study may not be representative of the disease prevalence since the majority of pastoralists encountered in the study area were not aware of the possibility for diagnosis and treatment of TB lymphadenitis at health facilities.

M. tuberculosis strains isolated in this study belonged to the E-A, CAS, EAI, and Beijing lineages. Epidemiologically, the most important type within the E-A lineage was SIT 149 (T3-ETH). This strain is isolated frequently in Ethiopia and among Ethiopian immigrants in Denmark (SpolDB4 (http://www.pasteur-guadeloupe.fr/tb/bd_myco.html); Brudey et al., 2006). The CAS lineage is primarily found in East Africa (including this study area;

SpolDB4; Groenheit et al., 2011), North India and Pakistan, reflecting intercontinental human migration (Gagneux et al., 2006; Gagneux & Small, 2007). Identification of strains of the EAI lineage among the pastoralists is interesting since this lineage has not yet been reported from the north and central Ethiopia. Its presence among the southern Ethiopian pastoralists may be explained by a closer contact with pastoral communities in Somalia and Kenya, where the EAI lineage has been described previously (SpolDB4; Brudey et al., 2006), rather than contacts of people from the northern and the central highlands of Ethiopia (Gutacker et al., 2006). Further investigation is required to determine the epidemiological significance of the Beijing strain noted in this study. In Africa, isolates of the Beijing lineage are most common in Southern Africa but occurs sporadically in East Africa as well (Groenheit et al., 2011). The *M. bovis* isolates from human pulmonary TB patients matched with both the dominant spoligotype of the animal isolates in the area (SB0133) and with SB0303, which has been isolated from cattle in central Ethiopia and in other countries of East Africa (Berg et al., 2011), thus indicating cattle-to-human transmission. All *M. bovis* isolates collected in this study had spacers 3 to 7 missing, a spoligotype feature that serves as a marker for strains belonging to the African 2 lineage of *M. bovis* that is highly prevalent in East Africa (Berg et al., 2011). It is therefore likely that the *M. bovis* isolates of this study belong to the African 2 lineage but further typing is needed for final categorization.

Animal isolates

The most common spoligotype pattern among the animal isolates was SB0133. Previously this spoligotype was reported as the second most dominant strain in Ethiopian cattle and it is a common type in East Africa (Berg et al., 2011; Biffa et al., 2010). The clustering rate of 0.3 of *M. bovis* in cattle found in this study, related to the observed prevalence of intradermal tuberculin test of 4.4% among Guji-Boran pastoralist cattle (Gumi et al., 2011), indicate ongoing endemic stable transmission of a dominant strain. Single isolates belonged to SB1942 and SB1983, which are new spoligotypes, whereas SB0933 was previously reported from France (Haddad et al., 2001). None of the isolates from goats could be identified as of the MTC. Other authors reported isolation of *M. bovis* and *M. tuberculosis* from goats slaughtered at the Mojo export abattoir in Ethiopia, however, their diagnosis were based only on colony morphology and discrimination by culture on growth media

with pyruvate or glycerol (Hiko & Agga, 2011). The low yield from gross lesions specimens in livestock is consistent with existing data from Ethiopia (Berg et al., 2009). This may be due to variable diagnostic capacity of meat inspectors or because of the presence of other granulomatous diseases in livestock.

The *M. tuberculosis* strain isolated from disseminated TB lesions in a camel belongs to the E-A lineage (SIT 149), a dominant strain in Ethiopia (Brudey et al., 2006). This is the first known report of *M. tuberculosis* from a camel in Ethiopia, indicating likely human to camel transmission. Isolation of *M. tuberculosis* from gross TB lesions was recently reported in Nigerian goats (Cadmus, 2009) and is more frequently found in Ethiopian cattle (Berg et al., 2009; Ameni et al., 2010). The close contacts between pastoralist communities and their livestock may be conducive for human to animal *M. tuberculosis* transmission, but further investigation is needed to determine the public health significance. We consider the finding of *M. tuberculosis* in camel as a rare event. In Ethiopia M. *tuberculosis* seems to be more frequently transmitted from humans to livestock than *M. bovis* from cattle to humans.

Non-Tuberculous Mycobacteria

Approximately 10% of the AFB positive isolates were characterized as NTM by molecular typing. Environmental mycobacteria are known to be opportunistic pathogens in HIV patients, but limited information is available for the bacterial isolates in this study. Previously reported were *M. fortuitum* in humans and livestock (Diguimbaye et al., 2006; Mawak et al., 2006; Berg et al., 2009; Tschopp et al., 2010b) and *M. flavescens* and *M. terrae* complex in wildlife in South-West Ethiopia (Tschopp et al., 2010b). The presence of *M. fortuitum* could indicate the presence of farcy in Ethiopia as it is difficult to distinguish it from *M. farcinogenes* (Diguimbaye et al. 2006). Its isolation from both animals and humans merits further investigation.

Epidemiology of *M. bovis* in South-Eastern Ethiopia

The parallel study on the prevalence of bTB in pastoral cattle herds in the Oromia region, southern Ethiopia (Gumi et al. 2011) indicated a true prevalence below 10%, which is in the range of endemically stable transmission in sedentary rural areas of Ethiopia (Tschopp et al. 2010a), but slightly higher to the Hamer area (Tschopp et al. 2010b). The transmission dynamics of sedentary rural and mobile pastoral cattle is likely similar, with

early exposure of calves, a variable latency period and transmission from adult cows through the airways and udder. This could explain the low level endemic transmission with high herd prevalence. Compared to sedentary rural communities, where we could not find human *M. bovis* (publication in preparation), cattle-human transmission seems effective in pastoral communities in South-Eastern Ethiopia. Hence there is likely a higher exposure of pastoralists to *M. bovis*, when compared to sedentary communities.

Conclusion

Identical spoligotypes of *M. bovis* isolates from humans and cattle, as well as collection of *M. tuberculosis* isolates from animals, indicates transmission between livestock, mainly between cattle and humans. Therefore, TB is of public health importance in pastoral settings of South-East Ethiopia and warrants locally adapted diagnosis and treatment protocols. *M. bovis* is naturally resistant to pyrazinamide, a commonly used treatment for TB. TB programs in areas where *M. bovis* is a potential etiologic agent in humans should therefore not neglect the zoonotic risk of bTB. *M. tuberculosis* isolates were represented by diversified lineages, requiring further typing to establish their position in the global TB population structure. This simultaneous study of mycobacteria in humans and livestock allowed relating transmission risks. It demonstrates an added value of a "One Health" approach of closer cooperation of human and animal health sectors.

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References

Addo K, Owusu-Darko K, Yeboah-Manu D, Caulley P, Minamikawa M, Bonsu F, et al. (2007) Mycobacterial Species Causing Pulmonary Tuberculosis at the Korle Bu Teaching Hospital, Accra, Ghana. Ghana Medical Journal 41 (2):52-57.

Ameni G, Desta F, Firdessa R (2010) Molecular typing of *Mycobacterium bovis* isolated from tuberculosis lesions of cattle in north eastern Ethiopia. Veterinary Record167 (4):138 -141.

Ameni G, Aseffa A, Engers H, Young D, Gordon S, Hewinson G et al. (2007) High Prevalence and Increased Severity of Pathology of Bovine Tuberculosis in Holsteins Compared to Zebu Breeds under Field Cattle Husbandry in Central Ethiopia. Clinical and Vaccine Immunology 14 (10):1356–1361.

Ayele WY, Neill SD, Zinsstag J, Weiss MG, Pavlik I (2004) Bovine tuberculosis: an old disease but a new threat to Africa. The International Journal of Tuberculosis and Lung Disease: The Official Journal of the International Union Against Tuberculosis and Lung Disease 8 (8): 924-37.

Berg S, Firdessa R, Habtamu M, Gadisa E, Mengistu A, Yamuah, et al. (2009) The Burden of Mycobacterial Disease in Ethiopian Cattle: Implications for Public Health. PLoS ONE4 (4):e5068.

Berg S, Garcia-Pelayo MC, Müller B, Hailu E, Asiimwe B, Kremer K, et al. (2011) African 2, a clonal complex of *Mycobacterium bovis* epidemiologically important in East Africa. Journal of Bacteriology 193 (3): 670-678.

Beyene D, Bergval I, Hailu E, Ashenafi S, Yamuah L, Aseffa A et al. (2009) Identification and genotyping of the etiological agent of tuberculous lymphadenitis in Ethiopia. The Journal of Infection in Developing Countries 3 (06): 412-419.

Biffa D, Skjerve E, Oloya J, Bogale A, Abebe F, Dahle U, et al.(2010) Molecular characterization of *Mycobacterium bovis* isolates from Ethiopian cattle. BMC Veterinary Research 6 (1): 28.

Brosch R, Gordon SV, Marmiesse M, Brodin P, Buchrieser C, et al. (2002) A new evolutionary scenario for the *Mycobacterium tuberculosis* complex. Proceedings of the National Academy of Sciences of the United States of America 99 (6): 3684-3689.

Brudey K, Driscoll JR, Rigouts L, Prodinger WM., Gori A, et al. (2006) *Mycobacterium tuberculosis* complex genetic diversity: mining the fourth international spoligotyping database (SpolDB4) for classification, population genetics and epidemiology. BMC Microbiology 6: 23.

Cadmus SI. (2009) *Mycobacterium bovis* and *M. tuberculosis* in Goats, Nigeria. Emerging Infectious Diseases 15 (12). Available from: <http://www.cdc.gov/eid/content/15/12/2066.htm> [Accessed 26 March 2011].

Comas I, Homolka, S, Niemann S, Gagneux S (2009) Genotyping of genetically monomorphic bacteria: DNA sequencing in *Mycobacterium tuberculosis* highlights the limitations of current methodologies. PloS One 4 (11):.e7815.

Cosivi O, Grange JM., Daborn CJ, Raviglione MC, Fujikura T, Cousins D, et al. (1998) Zoonotic tuberculosis due to *Mycobacterium bovis* in developing countries. Emerging Infectious Diseases 4 (1):59-70.

Demelash, B., Inangolet, F., Oloya, J., Asseged, B., Badaso, M., Yilkal, A. & Skjerve, E. (2009) Prevalence of Bovine tuberculosis in Ethiopian slaughter cattle based on post-mortem examination. Tropical Animal Health and Production 41 (5):755-765.

Diguimbaye-Djaïbe, C., V. Vincent, E. Schelling, M. Hilty, R. Ngandolo, H. H. Mahamat, G. Pfyffer, F. Baggi, M. Tanner, and J. Zinsstag. 2006. Species identification of non-tuberculous mycobacteria from humans and cattle of Chad. Schweiz.Arch.Tierheilkd. 148, no. 5:251-256.

Gagneux S & Small PM (2007) Global phylogeography of *Mycobacterium tuberculosis* and implications for tuberculosis product development. The Lancet Infectious Diseases 7 (5):328-337.

Gagneux S, DeRiemer K, Van T, Kato-Maeda M, de Jong BC, Narayanan S, et al. (2006) Variable host–pathogen compatibility in *Mycobacterium tuberculosis*. Proceedings of the National Academy of Sciences of the United States of America 103 (8):2869-2873.

Godreuil S, Jeziorski E, Banuls AL, Fraisse T, Van de Perre P, Boschiroli ML. (2010) Intrafamilial Cluster of Pulmonary Tuberculosis Due to *Mycobacterium bovis* of the African 1 Clonal Complex. Journal of Clinical Microbiology 48 (12): 4680-4683.

Groenheit R, Ghebremichael S, Svensson J, Rabna P, Colombatti R, et al. (2011) The Guinea-Bissau Family of *Mycobacterium tuberculosis* Complex Revisited. PloS One 6 (4): e18601.

Gumi B, Schelling E, Firdessa R, Aseffa A, Tschopp R, Yamuah L, et al.(2011) Prevalence of bovine tuberculosis in pastoral cattle herds in the Oromia region, southern Ethiopia. Tropical Animal Health and Production 43 (6):1081-1087.

Gutacker MM, Mathema B, Soini H, Shashkina E, Kreiswirth BN, Graviss EA, et al. (2006) Single-Nucleotide Polymorphism–Based Population Genetic Analysis of *Mycobacterium tuberculosis* Strains from 4 Geographic Sites. Journal of Infectious Diseases 193 (1):121 - 128.

Haddad N, Ostyn A, Karoui C, Masselot M, Thorel MF, Hughes SL, et al.(2001) Spoligotype diversity of *Mycobacterium bovis* strains isolated in France from 1979 to 2000. Journal of Clinical Microbiology 39 (10):3623-3632.

Hiko A, Agga GE (2011) First-time detection of mycobacterium species from goats in Ethiopia. Tropical Animal Health and Production 43 (1):133-139.

Idigbe EO, Anyiwo CE, Onwujekwe DI (1986) Human pulmonary infections with bovine and atypical mycobacteria in Lagos, Nigeria. The Journal of Tropical Medicine and Hygiene 89 (3):143-148.

Kamerbeek J, Schouls L, Kolk A, van Agterveld M, van Soolingen D, Kuijper S, et al. (1997) Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. Journal of Clinical Microbiology 35 (4):907-914.

Mamo G, Kassaye A, Sanni M, Ameni G (2009) A cross sectional study of camel tuberculosis in Ethiopia. Bulletin of Animal Health and Production in Africa,57 (1).

Mamo G, Bayleyegn G., Sisay T, Legesse M, Medhin G, Bjune G, et al. (2011) Pathology of camel tuberculosis and molecular characterization of its causative agents in pastoral regions of ethiopia. PloS One 6 (1):15862.

Mawak JD, Gomwalk NE, Bello CSS, Kandakai-Olukemi YT (2006) Human Pulmonary Infections with Bovine and Environment (Atypical) Mycobacteria in Jos, Nigeria. Ghana Medical Journal 40 (4): 132–136.

Michel AL, Müller B, van Helden PD (2010) *Mycobacterium bovis* at the animal-human interface: a problem, or not? Veterinary Microbiology 140 (3-4): 371-381.

Müller B. (2008) Molecular epidemiology and diagnosis of *Mycobacterium bovis* infections in African cattle. PhD Thesis, University of Basel 228 pp.

OIE (2009) Office International des Epizooties Terrestrial manual:World Health Organization for Animal Health, Paris. Office International des Epizooties.

Oloya J, Opuda-Asibo J, Kazwala R, Demelash AB, Skjerve E, Lund A, et al (2008) Mycobacteria causing human cervical lymphadenitis in pastoral communities in the Karamoja region of Uganda. Epidemiology and Infection 136 (5): 636-643.

Regassa A, Tassew A, Amenu K, Megersa B, Abunna F, Mekibib B, et al. (2010) Erratum to: A cross-sectional study on bovine tuberculosis in Hawassa town and its surroundings, Southern Ethiopia. Tropical Animal Health and Production 42 (5):1039.

Roberts GD, Koneman EW, Kim YK (1991) Mycobacterium. In: Balow A, ed. Manual of Clinical Microbiology. Washington D. C. pp. 304–339..

Shitaye JE, Tsegaye W, Pavlik I (2007) Bovine tuberculosis infection in animal and humanpopulations in Ethiopia: a review. Veterinarni Medicina 52 (8):317–332.

Tang C, Reyes JF, Luciani F, Francis AR, Tanaka MM (2008) spolTools: online utilities for analyzing spoligotypes of the *Mycobacterium tuberculosis* complex. Bioinformatics 24 (20): 2414 -2415.

Tschopp R, Schelling E, Hattendorf J, Young D, Aseffa A, Zinsstag J (2010a) Repeated cross-sectional skin testing for bovine tuberculosis in cattle kept in a traditional husbandry system in Ethiopia. The Veterinary Record 167 (7):250-256.

Tschopp R, Aseffa A, Schelling E, Berg S, Hailu E, Gadisa E, et al.(2010b) Bovine tuberculosis at the wildlife-livestock-human interface in Hamer Woreda, South Omo, Southern Ethiopia. PloS One 5 (8): e12205.

WHO (2008) Profiles of high-burden countries. Geneva, Switzerland, World Health Organization.

Zinsstag J, Schelling E, Bonfoh B, Fooks A, Kasymbekov J, Waltner-Toews D, Tanner M. (2009) Towards a “one health” research and application tool box. Veterinaria Italiana 45 (1): 121-133.

Zinsstag J, Schelling E, Roth F, Kazwala R (2006) Economics of Bovine Tuberculosis. In: *Mycobacterium bovis* infection in animals and humans. Boston, Wiley-Blackwell p.360.

12. Sero-prevalence of brucellosis and Q-fever in South-East Ethiopian pastoral livestock

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Abstract

To assess seroprevalences of brucellosis and Q-fever in pastoral livestock in southeast Ethiopia, cross-sectional study was carried out in three livestock species (cattle, camels and goats). Study was conducted from July 2008 to August 2010 in eight pastoral associations (PAs) from the selected districts were included in the study. Sera from a total of 1830 animals comprising 862 cattle, 458 camels and 510 goats were screened initially with Rose Bengal plate test (RBT) for brucellosis. All RBT positive and 25% of randomly selected negative sera were further tested by ELISA. These comprise a total of 460 animals (211 cattle, 102 camels and 147 goats). Out of sera from total 1830 animals 20% were randomly selected (180 cattle, 90 camels and 98 goats) and tested for Q-fever using ELISA. The seroprevalences of brucellosis was 1.4% (95% confidence interval (CI), 0.8-2.6), 0.9% (95% CI, 0.3-2.7) and 9.6% (95% CI, 5.2-17.1) in cattle, camels and goats, respectively. Goats and the older animal were at higher risk of infection (OR=7.3, 95% CI, 2.8-19.1) and (OR=1.7 95% CI, 0.9-2.9), respectively. Out of 98 RBT negative camel sera, 12.0% was positive for ELISA. The seroprevalences of Q-fever was 31.6% (95% CI, 24.7-39.5), 90.0% (95% CI, 81.8-94.7) and 54.2% (95% CI, 46.1-62.1) in cattle, camels and goats, respectively. We found positive animals for Q-fever test in all tested PAs for all animal species. Being camel and older animal was risk factor for infection (OR=19.0, 95% CI, 8.9-41.2) and (OR=3.6, 95% CI, 2.0-6.6), respectively. High seropositivity of Q-fever in all livestock species tested and higher seropositive in goats for brucellosis implies risks of human infection by both diseases. Thus, merit necessity of further study of both diseases in animals and humans in the area.

Keyword: Brucellosis, Q-fever, seroprevalence, pastoral livestock, southeast Ethiopia

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Introduction

Brucellosis is a disease of animals, especially livestock (cattle, goats, sheep, camels and pigs) but also wild animals. It is caused by bacteria of the genus *Brucella* spp. In livestock it is primarily a reproductive disease characterized by late abortion, retained foetal membranes, orchitis and impaired fertility (Zinsstag et al., 2011). *B. melitensis* is considered to have the highest zoonotic potential, followed by *B. abortus,* and *B. suis*. Brucellosis remains one of the most common zoonotic diseases worldwide with more than 500,000 human cases reported annually, particularly from developing countries (Boschiroli et al., 2001; Skalsky et al., 2008; Seleem et al., 2010).

The economic and public health impact of brucellosis remains of concern in developing countries (Roth et al., 2003). The disease poses a barrier to trade of animals and animal products, causes a public health hazard, and is an impediment to free animal movement (Zinsstag et al., 2011). In Africa and Central Asia, the incidence of brucellosis is generally considered higher in livestock raised in pastoral production systems (McDermott & Arimi, 2002), however, increasing intensified peri-urban production leads nowadays often to higher prevalence than in pastoral production systems.(Kebede et al., 2008). Brucellosis is endemic in humans and livestock in the Mediterranean region, Africa, the Near East, Central Asia and Central America (Pappas et al., 2006). Brucellosis in livestock and humans is re-emerging as a major epidemic in countries of the former Soviet Union (Roth et al., 2003).

In Ethiopia, serological studies of brucellosis have been carried out in farm animals. The presence in livestock varies between different parts of the country (Teshome et al., 2003; Berhe et al., 2007; Asmare et al., 2010; Megersa et al., 2010). Only few serological studies of brucellosis have demonstrated the occurrence of the disease among Borana and Hamer pastoralists; however these have highlighted the public health significance (Regassa et al., 2009).

Q-fever is a zoonotic disease caused by *Coxiella burnetii*. Livestock (cattle, sheep, camels and goats) are the main reservoirs of infection to humans(Kaabia & Letaief, 2009; Angelakis & Didier Raoult, 2010) It is also known as an occupational disease of veterinarians, farmers and abattoir workers(de Rooij et al., 2012) *Coxiella burnetii* the causative agent has been isolated from ticks. The transmission routes from livestock to

humans via respiratory route (Schelling et al., 2003). Infection in humans is often asymptomatic, but it can manifest as an acute disease (usually a self-limited flu-like illness, pneumonia or hepatitis) or as a chronic form (mainly endocarditis, but also hepatitis and chronic-fatigue syndrome). Q-fever is frequently misdiagnosed by physicians (Kaabia & Letaief, 2009). It is endemic both in livestock and humans in North and Sub-Saharan Africa (Kelly et al., 1993; Schelling et al., 2003; Steinmann et al., 2005; Mazyad & Hafez, 2007).

In Ethiopia, the existence of antibody against *C. burnetii* was reported in goats and sheep slaughtered at Addis Ababa abattoir and its peri-urban zone (Philip et al., 1966). A seroprevalence of 6.5% was also reported in Addis Ababa abattoir workers (Abebe, 1990). To our knowledge, there was no study on Q-fever in Ethiopian herds or in pastoral zones, where people live in very close contact to their livestock. Information on both diseases is scares in study zones. The objective of the present study was to assess the seroprevalences of brucellosis and Q-fever in pastoral livestock in southern Ethiopia and factors associated with seropositivity.

Materials and Methods

Study areas A cross-sectional study with a cluster sampling design was conducted from July 2008 to August 2010 in South Eastern Ethiopian pastoral zones of the Somali and Oromia regional states. The area was situated between the 39.21° and 41.24°E longitudes and 4.18–5.5°N latitude. Extensive pastoral livestock production is the main system and the basis of livelihood for millions of pastoralists in the study area. Climatic condition of the selected study areas is characterized by arid and semi-arid climate with bimodal rainfall pattern. Two districts were conveniently selected based on accessibility and security reasons from each of the two regional states. Liben and Filtu districts from Oromia and Somali Regional States were included. Eight pastoral associations (PAs) – each one of the cardinal directions per zonal capital were included in the study. These were Dhuko, Sirba, Arda-Bururi and Siminto PA`s from Oromia and Bifatu, Melkalibe, Hayadimtu and Bakaka PAs from Somali Regional State.

Sample size The sample size estimation considered clustering of animals within herds (Bennett et al., 1991), the sought precision of ±3% (standard error of 1.5%), assumed an intraclass correlation coefficient (rho) of 0.2 and an expected seroprevalence of 3% of

brucellosis. The total sample size calculated was 480 per species of animals per study site and a total of 128 herds. In Oromia, only cattle were sampled, whereas in Somali region, cattle, camels and goats were present for sampling. Twenty percent of total sample was tested for Q-fever.

Selection of Pastoral Households within PAs In each of the 8 PAs, fifteen animals per herd and species were selected randomly in eight herds. After discussion and agreement on procedures at the general PA meeting of each site, interested households were asked to register for participation. Using a list of registered households per PA as sampling frame, 8 households were selected with random numbers.

Sample Collection About 10ml of blood sample was collected from the jugular vein of each animal using plain vacutainer tubes and needles. Each sample was labelled with unique identification number. The tubes were kept overnight at room temperature to allow clotting of blood. The next morning sera was removed from the clot and stored in cryotubes at -20°C until analyses in the laboratory

Serology All sera were initially tested by Rose Bengal Test (RBT). For RBT 30µ of serum and 30µ of antigen (Rose Bengal stained *B. abortus* antigen obtained from BIO-RAD, Marnes-la-Coquette, France) were mixed and rotated on a glass plate for 4 min. Sera with no visible agglutination were recorded as negative while sera showing agglutination were considered positive. For further analysis, all RBT-positive and randomly selected 25% RBT-negative sera were tested by using ELISA kits for *Brucella abortus*. In addition, another randomly selected 20% of sera from 1830 total samples per species were tested for *Coxiella burnetii*. ELISA kit was obtained from IDEXX, Liebefeld-Bern Switzerland and tests were performed according to manufacturer's instructions. All samples and controls in the brucellosis and Q-fever ELISA were tested in duplicate and the mean OD values were used. Results were expressed as the percentage of the ratio between the sample OD and positive control OD (S/P-ratio) and were calculated as follows:

$$\frac{S}{P} = \frac{meanODsample - meanODnegativecontrol}{meanODpositivecontrol - meanODnegativecontrol} X100\%$$

The samples were considered seropositive for brucellosis if the percentage of the ratio was $\geq$ 80% and negative if lower; and for Q-fever seropositive if $\geq$ 40%; doubtful for values

between 30% and 40% and negative if < 30 %. These threshold-values for both tests were recommended by the manufacturers

Data analysis

The data was double-entered in Microsoft Access 2002 (Microsoft Corp., USA) and validated with EpiInfo version 3.3.2 before being imported to STATA 10/SE (STATA Corp., College Station, TX) for analysis We have used the xtgee model to determine the seroprevalence for each animal species while considering clustering within herds and to see if species, age category and sex were associated with sero-status.

Age categories were for cattle and goats <=1 years, 1-<=3 years and >3 years, and for camels <=2 years, 2-<=4 years and >4 years. Since not all sera were tested with the brucellosis ELISA, we present only results with the outcome of the RBT- binarily classified sero-status.

Results

Brucellosis seroprevalence A total of 862 cattle, 458 camels and 510 goats were tested for brucella anti-body, from 59, 32 and 34 herds, respectively. The sero-prevalences per species were 1.4% (95% CI, 0.8-2.6), 0.9% (95% CI, 0.3-2.7%) and 9.6% (95% CI, 5.2-17.1%) in cattle, camels and goats, respectively (Table12.1). In 10 out of 59 cattle herds and 5 of the 8 PAs, there was at least one positive animal. The three out of 32 camel herds and 2 of the 4 sampled PAs and 12 in 34 goat herds in all 4 sampled PAs were seropositive. Out of 98 RBT negative camel sera 12.0% was positive for ELISA. Univariable analysis of RBT showed that goats and older animals were at higher risk of infection (OR=7.3, 95% CI, 2.8-19.1) and (OR=1.7 95% CI, 0.9-2.9), respectively.

Table 12.1 Associations with risk factors for brucellosis seropositivity

Risk factors		Number of test negative	Number test positive (%)	Univariable OR (95% CI)
Species	Cattle	850	12 (1.4)	1
	Camel	454	4 (0.9)	0.7 (0.2-2.1)
	Goat	462	48 (9.6)	7.3(2.8-19.1)***
Age class	1[a]	295	4 (1.2)	1
	2[b]	772	32 (3.8)	1.4 (0.9-2.2)
	3[c]	699	28 (4.0)	1.7(0.9-2.9) *
Sex	Female	1408	63 (4.0)	1
	Male	358	1 (0.3)	0.3(0.2-0.4)***

* p<0.05; *** p<0.001

[a] Age categories, for cattle and goats <=1 years, and camels <=2 years, [b] for cattle and goats 1-<=3 years, and for camels 2-<=4 years, [c] for cattle and goats >3 years, and for camels >4 years.

Q-fever sero-prevalence For Q-fever, a total of 368 sera were tested for cattle, camels and goats for antibodies against Q-fever, where by the median of samples tested per herd was 3 for all species. The seroprevalences was 31.6% (95% CI, 24.7-39.5%), 90.0% (95% CI, 81.8-94.7%) and 54.2% (95% CI, 46.1-62.1%) in cattle, camels and goats, respectively (Table 12.2). We found positive animals in all tested PAs for all animal species. Being camel and older animal was risk factor for infection (OR=19.0, 95% CI, 8.9-41.2) and (OR=3.6, 95% CI, 2.0-6.6), respectively.

Table 12.2 Associations with risk factors for Q-fever seropositivity

Risk factors		Number test negative	Number test positive (%)	Univariable OR (95% CI)
Species	Cattle	123	57 (31.6)	1
	Camel	9	81 (90.0)	19.0(8.9-41.2)
	Goat	44	54 (54.2)	2.7(1.7-4.2)***
Age class	1[a]	43	17 (30.8)	1
	2[b]	77	77 (49.6)	2.2 (1.3-3.9)**
	3[c]	56	98 (62.7)	3.6(2.0-6.6)***
Sex	Female	140	170 (55.1)	1
	Male	36	22 (39.5)	0.6(0.4-1.1)

** p<0.01; *** p<0.001

[a] Age categories, for cattle and goats <=1 years, and camels <=2 years, [b] for cattle and goats 1-<=3 years, and for camels 2-<=4 years, [c] for cattle and goats >3 years, and for camels >4 years.

Discussions

Seroprevalence of brucellosis The RBT seroprevalence result of the present study is lower than many of the earlier reports. Seroprevalences as high as 38.7% and 22% have been reported from western and north-eastern parts of Ethiopia (Rashid, 1993) and (Sintaro, 1994), respectively. Slightly higher individual serological prevalences of 5.6%, 5.9%, 6.5%, 6.6%, 9.9% and 15.8% have also been recorded in Eritrea(Omer et al., 2000), Tanzania, Sudan, Chad, Kenya and Uganda, respectively (Kagumba & Nandokha, 1978; Hellman et al., 1984; Schelling et al., 2003; Faye et al., 2005). Differences in the seroprevalence observed in this study, as opposed to those recorded by previous researchers, may be due to differences in herd size, different management systems and the presence or absence of infectious foci, such as *Brucella*-infected herds, which could spread the disease among contact herds.

The seroprevalence of 9.6% of goats brucellosis in this study is inline with report of (Ashenafi et al., 2007) however, higher than the results of (Teklye & Kasali, 1989) from central Ethiopia, (Teshale et al., 2006) from Somali region, and (Megersa et al., 2010) from Borena pastoralist. Management could be a factor for lower prevalence reported by Teklye and Kasali since they studied goats under small holder mixed crop-livestock systems of central Ethiopia. The other investigators studies pastoral goats but used different tests and approaches. Zero seroprevalence was reported in goats from Chad

(Schelling et al., 2003) and Zambia (Muma et al., 2006). Difference in management system in different countries or absence of infected goat herds may attribute to such variations. In addition, brucellosis transmission is favored by a more humid climate, which prolongs the survival of the bacteria in the environment. However, our study sites were in a hot and dry region.

The seroprevalence of < 1% in camel is lower than previous reports from Borena pastoralists (Megersa et al., 2006; Megersa et al., 2010) and Jordan (Al-Majali et al., 2008). Comparable report was from Chad (Schelling et al., 2003). Inter-study variation may be due to difference in camel husbandry practices in different communities.

In our study ELISA found to be more sensitive than RBT to detect positive camels. This observation needs further evaluation of both tests to validate for diagnostic uses in camels.

The seroprevalence of brucellosis was higher in goats than in other two species of animals studied. It could be due to the highly contagious nature of the diseases in goats. The higher pathogenicity of *B. melitensis* and the close contact caused by the high density of the herds of goats, the intermixing of herds of different owners and heavy exposure in housing during the night can also contribute to this higher prevalence. The seroprevalence was also higher in females and older animals than in males and younger counterparts. This is in consent with the previous works (Mohammed et al., 2011). It has already been shown that susceptibility to brucellosis is greatest in sexually mature animals. Young animals are often resistant, although it should be noted that latent infections can occur and such animals may present a hazard when mature (Corbel, 2006).

Seroprevalence of Q-fever The seroprevalence of Q-fever found in this study is high in all the three animals species studied. The higher prevalence in camels is in agreement with previous reports from Chad (Schelling et al., 2003). But the seroprevalence in sera from cattle and goats are higher than previous report from Central African Republic (Nakouné et al., 2004) and Chad (Schelling et al., 2003). The highest seroprevalence observed in camels may be due to genetic susceptibility of camels to *C. burnetii* or host preference of tick vectors to camel. The high seroprevalence of anti Q-fever antibody in the present study may be due to ELISA kit used in our study is hyper-sensitivity to cross-react with related genera and species. However it needs further validation.

The high seroprevalence of Q-fever in all animal species studied and the higher prevalence of brucellosis in goats is particularly important finding that pinpoint the hazard to the health of the pastoralists. The importance of these zoonotic diseases in impairing the health of the community needs to be further studied in the future.

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Reference:

Abebe, A., 1990. Prevalence of Q fever infection in the Addis Ababa abattoir. Ethiopian Medical Journal 28 (3), 119–122.

Al-Majali, A.M., Al-Qudah, K.M., Al-Tarazi, Y.H., Al-Rawashdeh, O.F., 2008. Risk factors associated with camel brucellosis in Jordan. Tropical Animal Health and Production 40 (3), 193–200.

Angelakis, E. and Raoult, D., 2010. Q Fever. Veterinary microbiology 140 (3-4), 297–309.

Ashenafi, F., Teshale, S., Ejeta, G., Fikru, R., Laikemariam, Y., 2007. Distribution of brucellosis among small ruminants in the pastoral region of Afar, eastern Ethiopia. Revue Scientifique Et Technique (International Office of Epizootics) 26 (3), 731–739.

Asmare, K., Asfaw, Y., Gelaye, E., Ayelet, G., 2010. Brucellosis in extensive management system of Zebu cattle in Sidama Zone, Southern Ethiopia. African Journal of Agricultural Research, 5 (3), 252–263.

Bennett, S., Woods, T., Liyanage, W.M., Smith, D.L., 1991. A simplified general method for cluster-sample surveys of health in developing countries. World Health Statistics Quarterly. Rapport Trimestriel De Statistiques Sanitaires Mondiales 44 (3), 98–106.

Berhe, G., Belihu, K., Asfaw, Y., 2007. Seroepidemiological Investigation of Bovine Brucellosis in the Extensive Cattle Production System of Tigray Region of Ethiopia. International Journal of Applied Research in Veterinary Medicine 5 (2), 65–71.

Boschiroli, M.L., Foulongne, V., O'Callaghan, D., 2001. Brucellosis: a worldwide zoonosis. Current Opinion in Microbiology 4 (1), 58–64.

Corbel, M.., 2006. Brucellosis in humans and animals. World Health Organisation, Geneva, Switzerland.

Faye, B., Castel, V., Lesnoff, M., Rutabinda, D., Dhalwa, S., 2005. Tuberculosis and brucellosis prevalence survey on dairy cattle in Mbarara milk basin (Uganda). Preventive veterinary Medicine 67 (4), 267–281.

Hellman, E., Staak, C., Baumann, M., 1984. Bovine brucellosis among two different cattle populations in Bahr el Ghazal Province of Southern Sudan. Tropical Medical parasitology 35 (2), 123–126.

Kaabia, N. and Letaief, A., 2009. La fièvre Q en Tunisie. Pathologie Biologie 57 (5), 439–443.

Kagumba, M. and Nandokha, E., 1978. A survey of the prevalence of bovine brucellosis in East Africa. Bulletin of animimal Health and Production for Africa 26 (3), 224–229.

Kebede, T., Ejeta, G., Ameni, G., 2008. Seroprevalence of bovine brucellosis in smallholder farms in central Ethiopia (Wuchale-Jida district). Revue Méd Vét 159, 3–9.

Kelly, P.J., Matthewman, L.A., Mason, P.R., Raoult, D.,1993. Q fever in Zimbabwe. A review of the disease and the results of a serosurvey of humans, cattle, goats and dogs. South African Medical Journal = Suid-Afrikaanse Tydskrif Vir Geneeskunde 83 (1), 21–25.

Mazyad, S.A.M. and Hafez, A.O., 2007. Q fever (Coxiella burnetii) among man and farm animals in North Sinai, Egypt. Journal of the Egyptian Society of Parasitology 37 (1), 135–142.

McDermott, J.J. and Arimi, S.M., 2002. Brucellosis in sub-Saharan Africa: epidemiology, control and impact. Veterinary Microbiology 90 (1-4), 111–134.

Megersa, B, Molla, B., Yigezu, L., 2006. Seroprevalence of brucellosis in camels (Camelus dromedarius) in Borena Lowland, Southern Ethiopia Seroprevalence de la brucellose chez les dromadaires (Camelus dromedarius) dans la plaine de Borena au Sud de l'Ethiopie [Internet]. Available from: <http://www.ajol.info/index.php/bahpa/article/view/32718> [Accessed 15 February 2011].

Megersa, B., Biffa, D., Abunna, F., Regassa, A., Godfroid, J., Skjerve, E., 2010. Seroprevalence of brucellosis and its contribution to abortion in cattle, camel, and goat kept under pastoral management in Borana, Ethiopia. Tropical Animal Health and Production 43 (3), 651–656.

Mohammed, F.U., Ibrahim, S., Ajogi, I., Olaniyi, B.J.O., 2011. Prevalence of Bovine Brucellosis and Risk Factors Assessment in Cattle Herds in Jigawa State. ISRN Veterinary Science 2011, 1–4.

Muma, J.B., Samui, K.L., Siamudaala, V.M., Oloya, J., Matop, G., Omer, M.K., Munyeme, M., Mubita, C., Skjerve, E., 2006. Prevalence of antibodies to Brucella spp. and individual risk factors of infection in traditional cattle, goats and sheep reared in

livestock-wildlife interface areas of Zambia. Tropical Animal Health and Production 38 (3), 195–206.

Nakouné, E., Debaere, O., Koumanda-Kotogne, F., Selekon, B., Samory, F., Talarmin, A. ,2004. Serological surveillance of brucellosis and Q fever in cattle in the Central African Republic. Acta Tropica 92 (2), 147–151.

Omer, M.K., Skjerve, E., Holstad, G., Woldehiwet, Z., MacMillan, A.P., 2000. Prevalence of Antibodies to Brucella spp. in Cattle, Sheep, Goats, Horses and Camels in the State of Eritrea; Influence of Husbandry Systems. Epidemiology and Infection 125 (2), 447–453.

Pappas, G., Papadimitriou, P., Akritidis, N., Christou, L., Tsianos, E.V., 2006. The new global map of human brucellosis. The Lancet Infectious Diseases 6 (2), 91–99.

Philip, C.B., Hoogstraal, H., Reiss-Gutfreund, R., Clifford, C.M., 1966. Evidence of rickettsial disease agents in ticks from Ethiopian cattle. Bulletin of the World Health Organization 35 (2), 127–131.

Rashid, M., 1993. Reproductive wastage in cattle due to bovine brucellosis. In: Livestock Improvement Conference. Addis Ababa, Ethiopia, Ethiopian Institute of Agricultural Research, 270–272.

Regassa, G., Mekonnen, D., Yamuah, L., Tilahun, H., Guta, T., Gebreyohannes, A., Aseffa, A., Abdoel, T., Smits, H., 2009. Human Brucellosis in Traditional Pastoral Communities in Ethiopia. International Journal of Tropical Medicine 4 (2), 59–64.

de Rooij, M.M.T., Schimmer, B., Versteeg, B., Schneeberger, P., Berends, B.R., Heederik, D., van der Hoek, W., Wouters, I.M., 2012. Risk factors of Coxiella burnetii (Q fever) seropositivity in veterinary medicine students. PloS one 7 (2), e32108.

Roth, F., Zinsstag, Jakob, Orkhon, D., Chimed-Ochir, G., Hutton, G., Cosivi, O., Carrin, G., Otte, J., 2003. Human health benefits from livestock vaccination for brucellosis: case study. Bulletin of the World Health Organization 81 (12), 867–876.

Schelling, E., Diguimbaye, C., Daoud, S., Nicolet, J., Boerlin, P., Tanner, M., Zinsstag, J., 2003. Brucellosis and Q-fever seroprevalences of nomadic pastoralists and their livestock in Chad. Preventive Veterinary Medicine 61 (4), 279–293.

Seleem, M.N., Boyle, S.M., Sriranganathan, N., 2010. Brucellosis: a re-emerging zoonosis. Veterinary Microbiology 140 (3-4), 392–398.

Sintaro, T., 1994. The impact of brucellosis on productivity in an improved dairy herd of Chaffa state farm, Ethiopia. Master of Science thesis. Berlin, Germany., Free University of Berlin.

Skalsky, K., Yahav, D., Bishara, J., Pitlik, S., Leibovici, L., Paul, M., 2008. Treatment of human brucellosis: systematic review and meta-analysis of randomised controlled trials. BMJ 336 (7646), 701 –704.

Steinmann, P., Bonfoh, B., Péter, O., Schelling, E., Traoré, M., Zinsstag, J., 2005. Seroprevalence of Q-fever in febrile individuals in Mali. Tropical Medicine & International Health 10 (6), 612–617.

Teklye, B. and Kasali, O., 1989. The prevalence of brucellosis in small ruminants in central Ethiopia. Bulletin of Animal Health and Production for Africa (37), 97–98.

Teshale, S., Yibeltal, M., Abaineh, D., Awoke, K., 2006. Seroprevalence of small ruminant brucellosis in selected districts of Afar and Somali pastoral areas of eastern Ethiopia: the impact of husbandry practice. Revue Médicine véteterinaiane 157 (11), 557–563.

Teshome, H., Molla, B., Tibbo, M., 2003. A Seroprevalence Study of Camel Brucellosis in Three Camel-rearing Regions of Ethiopia. Tropical Animal Health and Production 35 (5), 381–390.

Zinsstag, J, Schelling, E., Solera, X., Blasco, J., Moriyon, I., 2011. Brucellosis. In: Oxford Textbook of Zoonoses, 54–64.

13: General discussion and conclusions

13.1 Tuberculosis in south Ethiopian pastoralists and their livestock

This study was undertaken by multi-disciplinary team approach with integration of different work packages by involving veterinarian, physician, epidemiologist, molecular biologist and laboratory technologists. The work components were includes PPD skin testing of cattle, camels and goats in the field and TB suspected samples collection from cattle, camels and goats at abattoir and sputum and FNA at hospitals in the study area. In addition serology was done for brucellosis and Q-fever.

13.1.1 BTB in livestock

Previous studies have shown that BTB is endemic in Ethiopian cattle with highest prevalence recorded in crossbreed animals in central Ethiopia [1]. Different abattoir surveys based on gross pathology revealed that prevalence was range from 3.5% to 5.2% [2–5]. Prevalence of PPD skin test reactor cattle in the present study in south-east Ethiopian pastoralists were 4.0 % and 5.4% at 4 mm and 2 mm cut-off value used for interpretation, respectively. This result is comparable with report from Ethiopia [6–8], Uganda [9] and Zambia [10]. When prevalence aggregated to study regions we observed low prevalence in Somali region, 2% and 3.3 % at 4 mm and 2 mm cut-off, respectively as compared to Oromia region, which was relatively higher, 4.4% and 6.1%. Prevalence in camels and goats were < 1% in our study. In cattle we observed high BTB prevalence in some localized sites, namely; Arda-Bururi and Siminto in Oromia region and Hayadimitu in Somali region as hot spot village as it described in chapter 9 and 10. Tschopp et al [11] was also reported similar observation in traditionally kept zebu cattle in three Districts. We do not have an explanation why certain localities have high reactor animals while animals in other sites have low or zero prevalence without animal movement restriction between PAs. Many classical risk factors were not associated with PPD skin test positivity in this study.

The parallel abattoir survey revealed that 0.95% of inspected cattle were harboring TB suspected lesion with relatively high (72%) AFB positive culture yield. Abattoir prevalence in cattle in present study is lower than Berg et al [3]. In camels and goats 12.0 % and 4.4% had TB suspected lesion, respectively. However only 4.0% in camels and 11.8% in goats of

these lesions yielded AFB positive cultures. Only one single isolate from camel was MTC (*M. tuberculosis*) and none of isolate from goats was MTC. Tschopp et al[12] reported similar results from Hamer goats in South-West Ethiopia. Previously *M. tuberculosis* isolation from TB suspected lesions were reported in Nigerian goats [13] and cattle in Ethiopia[3,14]. Higher tuberculin skin test reactors in camels and goats were reported from Afar pastoralists in North-East Ethiopia (personal communication).

BTB prevalence of 5.0% and 4.2% in camels and goats, respectively were reported based on abattoir survey. These surveys were based on camels slaughtered at Dire Dawa abattoir in eastern Ethiopia [15] and goats at Mojo export abattoir in central Ethiopia [16].

In other countries 2.4% of prevalence in goats from Pakistan [17], 4.47% in Nigerian goats [13], and *M. caprea* was reported from European goats[18]. *M. bovis* was also reported in previous studies from camels in Mauritania [19] and in United Arab Emirates [20, 21].

About 10.4% of PPD skin tested camels were reactors to avian PPD whereas reactors cattle and goats were lower in our study. In parallel abattoir survey we also observed higher TB like lesions in camels than cattle and goats with low AFB positive culture yield. This may explain that higher avian PPD reactors in camels are due to cross-reaction with related genera.

BTB infection causes milk and meat productivity losses to livestock owners in addition to decreased value of the slaughter animal if condemned during meat inspection. Close contact to animals, consumption of raw milk and pooling of un-pasteurized milk from different animals (annex 1c) for home consumption and marketing are common practices among pastoral communities in the study area, which entail the risk of zoonotic transmission of tuberculosis. Moreover, the study area has the highest cattle population densities in the country and is a major source of livestock for domestic and export market. Unofficial cross-border livestock trading to the neighboring countries is also common practices in the area. Knowing the BTB epidemiology in such pastoralist zones is important in regard of the possible public health implications, and the impact of the disease on livelihood and economy of pastoralists.

Some factors which may needs further discussion and investigation are;

1. **Breed factor**: Ameni et al 2007 [1] reported significant association between local zebu and exotic breed in susceptibility to BTB in central highlands of Ethiopia. In

our study all cattle breeds were local zebu of Boran breed and Guji cattle or cross-breed of the two. In Somali region almost all tested cattle were Boran breed. In present study we didn't observe significant difference in local zebu breed in susceptibility to BTB. Tschopp et al [11] was also reported similar findings in local zebu. BTB genetic susceptibility study by Trinity College as the part of work pakege three of Wellcome Trust project expected to come up with the possible resistance traits in Ethiopian cattle

2. **Management practices**: Pastoralists in the study areas were kept their livestock on communal pasture land during day where all types of livestock intermingled, and in the night kept in the open kernel. It is difficult to identify difference in management and almost all the pastoralist in the area manages their livestock in similar management practice. In Oromia region we observed significant difference in prevalence between herds' drinking water from river and stagnant water sources during the main dry season, which may be due to aggregation of large number of different livestock from different pastoral households around limited watering points facilitating BTB transmission either directly between animals or by contaminated pastures and water sources. It could need further investigation.
3. **Species of animals**: In our study BTB prevalence in camels and goats was less than 1%. This could be due to inter-species difference in genetic susceptibility. Thus, camels and goats would play limited role in serving as reservoir host for BTB in the area. Secondly, we used PPD skin test protocol designed for cattle which may not be appropriate for camels and goats.
4. **Inter-regional differences**: We observed relatively higher BTB reactors in Oromia region than Somali region. Study area in Somali pastoralists was more arid and drier zone than Oromia pastoralists, which was consists of pastoralist and agro-pastoralists under arid and semi-arid zones. This semi-arid zone may play role in maintaining mycobacteria in environment than harsh drier zone of Somali region
5. **Environmental Mycobacteria**: About 10.4% of tested camels were reactors to avian PPD. It is inline with a report of 10.0 % [22] and 11% [7] in cattle from Zambia and Ethiopia, respectively. However, in contrast to our result, these studies also in cattle and goats showed relatively high proportions of avian PPD reactors. The

parallel abattoir survey also support field result that 12.0% of inspected camels harbors TB like lesion but low yield (4.0%) of AFB positive mycobacteria. All livestock groups are kept and pastured together in the field. The observed differences might be due to different in susceptibility to NTM mycobacteria. Other mycobacteria related genera may produce TB like granulomatous lesion in camels. Its impact on camel production needs further investigation.

6. **Meat inspectors' ability to differentiate TB lesions**: TB suspected sample collection was undertaken by different trained meat inspectors at different abattoir. Less than 1% of cattle investigated at Negelle abattoir was positive for TB with high yield of AFB (72.0%) positive culture. This result is lower than previous study conducted in various sites [3]. On the other hands higher proportion of camels and goats inspected during study period was positive for gross lesion but very low AFB positive culture yield. As stated in Berg et al (2009) this variation may be due to ability of meat inspectors in identifying TB lesions or difference in species susceptibility to mycobacteria or other genera.
7. ***M. tuberculosis* in camels**: Human TB strain isolated from camel was belonging to dominant TB lineage in Ethiopia as described in [23] SIT 149 (T3-ETH). Possible explanation was described in chapter 11.
8. **Animal density:** Ethiopia has more than 82 million people[24] and highest livestock population in the region, 52 million cattle, 63 million sheep and goats, 2.5 million camels and 42 million poultry[25]. To satisfy growing milk demand the urban/ backyard dairy farming is common practice in towns [26]. In this regard dairy owners' family share shelter in a confined place. This could increase contact between animals and humans more frequently and closely than pastured animals and facilitate TB transmission between human and animals. A future research should consider a role of animal density and contact rate in maintaining TB prevalence and transmission in the urban area.

In conclusion BTB prevalence in local zebu in pastoral area is low in general and less than 1% in camels and goats. However, many factors may influence the test results. Additional assessment, which covers wider area, is required to generalize the result.

13.1.2 BTB in human

Among 173 isolates from sputum and FNA that was characterized by molecular typing 160 were confirmed as *M. tuberculosis*, three as *M. bovis*, and the remaining 10 were typed as NTMs. All FNA isolates identified as *M. tuberculosis* and all *M. bovis* we identified were from pulmonary TB. Cross-sectional study of human lymph node TB in Ethiopia under work pakege two was also reported similar result (unpublished report)

Recently the cases of NTM in pulmonary TB are increasing [27,28]. They are known to be opportunistic pathogen in HIV/AIDS patients, but we didn't investigate status of HIV in our study participants due ethical concerns.

In conclusion, the role of *M. bovis* in TB lymphadenitis is limited and only 2% of pulmonary TB in study area was due to *M. bovis*. Thus, isolation of *M. bovis* from humans and *M. tuberculosis* from livestock as described in above section suggests limited transmission between livestock and humans in the pastoral areas of southeast Ethiopia.

13.2 Options for TB control in humans and animals

In the present study PPD skin test revealed relatively low prevalence of BTB in pastoral livestock, 4.0%, 0.4% and 0.2 % in cattle, camels and goats, respectively. In abattoir survey 0.95%, 12.0% and 4.4% of inspected cattle, camels and goats had suspected TB lesions, respectively. However out of AFB positive cultures, MTC were 78%, 33% and 0% in cattle, camels and goats, respectively. In contrary study in intensive dairy farms around Addis Ababa showed high prevalence up to 90.0% positive animals (unpublished report) and Berg et al (2009) reported relatively higher abattoir prevalence. We isolate *M. tuberculosis* from TB lesion in camel and *M. bovis* from pulmonary TB patients in the study area.

Although over all prevalence was low in pastoral setting some hot spot was observed reaching up to 10.0% of PPD skin test reactor and local area specific control strategies is needed.

Low prevalence observed in camels and goats may reflect that role of this animals serving as BTB reservoirs might be minimal. Tschopp et al [12,29] in her survey of BTB in Ethiopian wildlife detected no *M. bovis*. The role of wildlife in maintaining *M. bovis* is unknown. Therefore, control strategies should focus on cattle with special attention to regional differences. The result of work package six is expected to come up with estimation of the

costs to society of BTB, and cost-benefit model of intervention and appropriate control strategy proposal.

Increased demand for milk production may change current traditional production system through intensification which may change the current prevalence status. Therefore, regular surveillance should be in place with special attention to hot spot area to prevent further spread. In such focal area BCG vaccination of calves could be envisaged as alternative control strategy since it demonstrate 70.0% protection against bovine TB in efficacy trial [30].We didn't observe significant difference between Boran breed and Guji-cattle in susceptibility to PPD reaction. Therefore, improving milk production of local Zebu (Boran breed) to limit importation of exotic breed to the area should be considered in the control program.

Spoligotype pattern of *M. bovis* isolated from PTB patient matched with *M. bovis* from cattle. *M. tuberculosis* from camel is also matched with dominant *M. tuberculosis* spoligotype pattern in human in Ethiopia. This confirms transmission between animals and humans. This could be due to closed contact between human and livestock, raw milk and meat consumption habit. Therefore, control method that aim at change lifestyle and feeding habits should be target at different ethnic and age groups. For example raw meat consumption habit is common practice in Guji pastoralist but not in Boran and Somali pastoralist. Therefore risk of getting infected via raw meat consumption will be high in Guji. Awareness creation will be appropriate for them. Awareness creation on change in cultural nutritional habits should aim at pastoral children. Appropriate treatment regime and diagnostic facilities are needed for *M. bovis* infected patients.

13.3 Brucellosis and Q-fever in southeast Ethiopian pastoral livestock

RBT test result revealed that prevalence of brucellosis was 1.4%, 0.9% and 9.6% in cattle, camels and goats, respectively. Twelve percent (12.0%) of RBT negative camel sera were positive for ELISA. The prevalence of Q-fever was 32%, 90% and 54.2% in cattle, camels and goats, respectively. Earlier reports by Rashid [31] and Sintaro [32] were presented higher prevalence of brucellosis in Ethiopia and other countries of Africa [33–36]. The prevalence of Q-fever is high in all three animals species studied. The high prevalence of Q-fever was

also reported in previous studies from Chad [35] and Central African Republic [37]. Possible explanation for observed variation in this study was described in chapter 12.

Although there was no control program at present, knowing the status of both brucellosis and Q-fever is important for the implementation of future control strategies. The close contact between animals and pastoralists, consumption of un-pasteurized dairy products and meat implies risk of infection by both diseases. The high prevalence of Q-fever in all animal species studied and the higher prevalence of brucellosis in goats is particularly important finding that identify the hazard to the health of the pastoralists. The importance of these zoonotic diseases in impairing the health of the community needs to be further studied in the future.

13.4 Public engagement and policy dialogue

Evidences from several initiatives show a lacking connection between research and development action. Response to the actual needs of society is delayed. Research fails to reach the level of policy making and the development of effective intervention. On the other hand policy problems are not taken up by research.[38]. Examples of such initiatives are advocating evidence brokerage for health policy in East Africa or National Competence Centre for Research North-South (NCCR North-South) on research for health services to nomadic pastoralists in Sahelian countries of Africa through a trans-disciplinary process by NCCR. Continuous dialogue between researchers, the concerned population and government authorities validate iterative cycles of research and pilot interventions leading to the formulation of a new integrated policy for nomadic pastoralists in Chad[38].

In Ethiopia poor understanding of the pastoral way of life among the policy makers in the past has led to the exclusion of pastoralists' issues from policy development. They consider pastoralism as a backward system that needs to be changed. Such perceptions are generally ill-suited to the effective use of the pastoral areas and their way of life. Consequently, Ethiopian pastoralists have experienced policies and socio-political exclusion. However pastoralist development is important for the sustainable growth of the Ethiopian economy and the achievement of the Millennium Development Goals (MDGs) [39]. On the other hands the country's priority is market oriented production system, and to increase exports of meat and livestock to benefit Ethiopian livestock producers and exporters and to promote national economic development [25]. In this regard a big contribution is expected from

pastoral areas. To achieve this objective appropriate diseases control intervention strategies that includes all stakeholders should be in place. During the Wellcome Trust project series of stakeholders meeting was initiated and hosted. They involved livestock holders, veterinary and public health authorities and decision makers, which resulted in a series of dialogues with officials on how to reduce BTB in the future. Such dialogues should be extended to the community level for the future planning of appropriate intervention strategies.

13.5 Message and recommendation of this thesis

1. This thesis provided baseline information on BTB in pastoral livestock in southeast Ethiopia. Low bovine TB prevalence in pastoral livestock in general and < 1% prevalence in camels and goats. To satisfy a milk demand of a fast growing population in the area, any future planning of importing high milk producing exotic breed should be handled with care and improving local breeds may be a better option. Furthermore we observed regional and local area variation in prevalence with some villages as hot spot. Risk factors for such variation should further studied to develop locally adapted control strategies.
2. In the present study we tested camels and goats using the protocol developed for cattle to perform PPD skin testing. The use of this protocol in camel and goat should be optimized in the future study.
3. We were able to isolate *M. bovis* from human PTB patients and *M. tuberculosis* from an animal. Thus, we confirm transmission between livestock and humans in pastoral areas. Therefore, tuberculosis is of public health importance in pastoral settings of southeast Ethiopia and warrants locally adapted control strategies. Particular attention should be given to TB treatment programs in areas where *M. bovis* is a potential etiologic agent in human pulmonary TB.
4. About 10.0% of AFB positive isolates were characterized as NTM. The role of NTM in clinical TB both in animals and humans merits further investigation.
5. Both brucellosis and Q-fever are prevalent in the study area, the close contact between animals and pastoralists, consumption of raw milk and meat implies risk of infection by both diseases. The high prevalence of Q-fever in all animal species studied and the higher prevalence of brucellosis in goats is particularly important

finding that pinpoint the risk to the health of the pastoralists. Significance of these zoonotic diseases in impairing the health of the community needs to be further studied in the future.

6. During collaborative work between North and South partners; the North partners should understand practical field work difficulties in the south. E.g I would like to mention here what I faced in Somali region. We did PPD skin testing in a village and on the day of result reading, because of raining in the night, we could not travel to the site by car and decided to walk on foot 42km in one day to respect community appointment and to accomplish planned activities on time.

Reference:

1. Ameni, G. *et al.* High Prevalence and Increased Severity of Pathology of Bovine Tuberculosis in Holsteins Compared to Zebu Breeds under Field Cattle Husbandry in Central Ethiopia. *Clin Vaccine Immunol.* **14**, 1356–1361 (2007).
2. Shitaye, J.., Tsegaye, W. & Pavlik, I. Bovine tuberculosis infection in animal and humanpopulations in Ethiopia: a review. *Veterinarni Medicina* **52**, 317–332 (2007).
3. Berg, S. *et al.* The Burden of Mycobacterial Disease in Ethiopian Cattle: Implications for Public Health. *PLoS ONE* **4**, e5068 (2009).
4. Demelash, B. *et al.* Prevalence of Bovine tuberculosis in Ethiopian slaughter cattle based on post-mortem examination. *Tropical Animal Health and Production* **41**, 755-765 (2009).
5. Regassa, A. *et al.* Erratum to: A cross-sectional study on bovine tuberculosis in Hawassa town and its surroundings, Southern Ethiopia. *Tropical Animal Health and Production* **42**, 1039 (2010).
6. Ameni, G., Amenu, K. & Tibbo, M. Prevalence and Risk Factor Assessment in Cattle and Cattle Owners in Wuchale-Jida District, Central Ethiopia. *The International Journal of Applied Research in Veterinary Medicine* **1**, 17-25 (2003).
7. Fetene, T. & Kebede, N. Bovine tuberculosis of cattle in three districts of northwestern Ethiopia. *Tropical Animal Health and Production* **41**, 273-277 (2009).
8. Tschopp, R., Schelling, E., Hattendorf, J., Aseffa, A. & Zinsstag, J. Risk factors of bovine tuberculosis in cattle in rural livestock production systems of Ethiopia. *Preventive Veterinary Medicine* **89**, 205-211 (2009).

9. Bernard, F., Vincent, C., Matthieu, L., David, R. & James, D. Tuberculosis and brucellosis prevalence survey on dairy cattle in Mbarara milk basin (Uganda). *Preventive Veterinary Medicine* **67**, 267-281 (2005).
10. Munyeme, M. *et al.* Risk factors associated with bovine tuberculosis in traditional cattle of the livestock/wildlife interface areas in the Kafue basin of Zambia. *Prev Vet Med* **85**, 317-28 (2008).
11. Tschopp, R. *et al.* Repeated cross-sectional skin testing for bovine tuberculosis in cattle kept in a traditional husbandry system in Ethiopia. *Vet. Rec* **167**, 250-256 (2010).
12. Tschopp, R. *et al.* Bovine tuberculosis at the wildlife-livestock-human interface in Hamer Woreda, South Omo, Southern Ethiopia. *PLoS ONE* **5**, e12205 (2010).
13. Cadmus, S.I. Mycobacterium bovis and M. tuberculosis in Goats, Nigeria. *Emerg. Infect. Dis.* **15**, (2009).
14. Ameni, G., Desta, F. & Firdessa, R. Molecular typing of Mycobacterium bovis isolated from tuberculosis lesions of cattle in north eastern Ethiopia. *Veterinary Record* **167**, 138 -141 (2010).
15. Mamo, G., Kassaye, A., Sanni, M. & Ameni, G. A cross sectional study of camel tuberculosis in Ethiopia. *Bulletin of Animal Health and Production in Africa* **57**, (2009).
16. Hiko, A. & Agga, G.E. First-time detection of mycobacterium species from goats in Ethiopia. *Trop Anim Health Prod* **43**, 133-139 (2011).
17. Javed, M.T. *et al.* Percentage of reactor animals to single comparative cervical intradermal tuberculin (SCCIT) in small ruminants in Punjab Pakistan. *Acta Tropica* **113**, 88-91 (2010).
18. Aranaz, A., Cousins, D., Mateos, A. & Dominguez, L. Elevation of Mycobacterium tuberculosis subsp. caprae Aranaz et al. 1999 to species rank as Mycobacterium caprae comb. nov., sp. nov. *Int J Syst Evol Microbiol* **53**, 1785-1789 (2003).
19. Chartier, F., Chartier, C., Thorel, M.F. & Crespeau, F. [A new case of Mycobacterium bovis pulmonary tuberculosis in the dromedary (Camelus dromedarius) in Mauritania]. *Rev Elev Med Vet Pays Trop* **44**, 43-47 (1991).

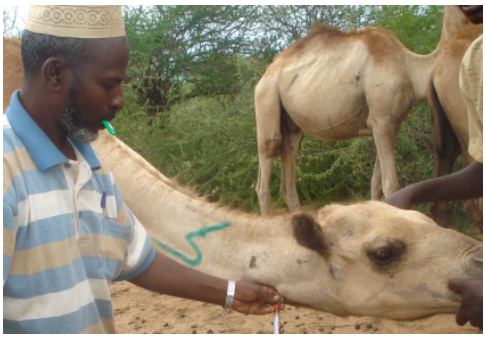
20. Kinne, J. *et al.* Camel tuberculosis--a case report. *Trop Anim Health Prod* **38**, 207-213 (2006).
21. Wernery, U. *et al.* Tuberculosis outbreak in a dromedary racing herd and rapid serological detection of infected camels. *Veterinary Microbiology* **122**, 108-115 (2007).
22. Shirima, G.M., Kazwala, R.R. & Kambarage, D.M. Prevalence of bovine tuberculosis in cattle in different farming systems in the eastern zone of Tanzania. *Prev Vet Med* **57**, 167-72 (2003).
23. Brudey, K. *et al.* Mycobacterium tuberculosis complex genetic diversity: mining the fourth international spoligotyping database (SpolDB4) for classification, population genetics and epidemiology. *BMC Microbiol.* **6**, 23 (2006).
24. PRB PRB 2011 World population Data Sheet. (2011).
25. Gebre Egziabhe, B. Animal Health Services delivery in Ethiopia. *PROCEEDINGS OF THE CAHNET-ETHIOPIA LAUNCHING WORK SHOP HELD AT GHION HOTEL* 10-13 (2010).
26. Kurwijila, L., Henriksen, J., Aboud, A. & Kifaro, G. Strategies for market orientation of small scale milk producers and their organisations. *Proceedings of a worshop held at Mogororo Hotel, Morogoro. Tanzania, 20-24 March 1995* (1995).
27. Woodring, J.H. & Vandiviere, H.M. Pulmonary disease caused by nontuberculous mycobacteria. *J Thorac Imaging* **5**, 64-76 (1990).
28. Johnson, M.M., Waller, E.A. & Leventhal, J.P. Nontuberculous mycobacterial pulmonary disease. *Curr Opin Pulm Med* **14**, 203-210 (2008).
29. Tschopp, R. *et al.* Bovine tuberculosis in Ethiopian wildlife. *J. Wildl. Dis* **46**, 753-762 (2010).
30. Ameni, G., Vordermeier, M., Aseffa, A., Young, D. & Hewinson, G. Field evaluation of the efficacy of Mycobacterium bovis bacillus Calmette-Guerin against bovine tuberculosis in neonatal calves in Ethiopia. *Clinical and Vaccine Immunology* **17**, 1533-1538 (2010).
31. Rashid, M. Reproductive wastage in cattle due to bovine brucellosis. *Livestock Improvement Conference* **4**, 270-272 (1993).

32. Sintaro, T. The impact of brucellosis on productivity in an improved dairy herd of Chaffa state farm, Ethiopia. (1994).
33. Kagumba, M. & Nandokha, E. A survey of the prevalence of bovine brucellosis in East Africa. *Bulletin of animimal Health and Production for Africa* **26**, 224-229 (1978).
34. Hellman, E., Staak, C. & Baumann, M. Bovine brucellosis among two different cattle populations in Bahr el Ghazal Province of Southern Sudan. *Tropical Medical parasitology* **35**, 123-126. (1984).
35. Schelling, E. *et al.* Brucellosis and Q-fever seroprevalences of nomadic pastoralists and their livestock in Chad. *Preventive Veterinary Medicine* **61**, 279-293 (2003).
36. Faye, B., Castel, V., Lesnoff, M., Rutabinda, D. & Dhalwa, S. Tuberculosis and brucellosis prevalence survey on dairy cattle in Mbarara milk basin (Uganda). *Preventive veterinary Medicine* **67**, 267-281. (2005).
37. Nakouné, E. *et al.* Serological surveillance of brucellosis and Q fever in cattle in the Central African Republic. *Acta Tropica* **92**, 147-151 (2004).
38. Zinsstag, J. Animal health research. *Science* **315**, 1193 (2007).
39. PFE *Pastoralist perspectives of poverty reduction strategy program of Ethiopia: experiences and lesson from Afar Region of Ethiopia*. (Pastoralist Forum Ethiopia.: Addis Ababa, Ethiopia, 2009).

14. Annexes

Annex 1. Field work Photos

a) PPD skin testing (Photo: Balako)



b) Blood sample collection (Photo: Balako)

c) Pooled milk market at Dhuko PA in Oromia region (Photo: Balako)

d) Multi-purpose house (butcher, residence & local night club) (photo: Balako)

e) Camel milk present from Somali Pastoralist elder (Photo: Balako)

f) Unsafe drinking water, Somali pastoralist women (photo: Balako)

Annex 2. Curriculum vitae

Name:	**Balako Gumi Donde**
Date of Birth:	October 19, 1972
Place of Birth:	Liben, Negelle, Ethiopia
Sex	Male
Marital Status	Married to Banchiliyew Yohannes
Nationality	Ethiopian
Children	Two sons (Horo and Anole)

Language proficiency

Oromifa, English and Amharic speak, read & write

Current address

Bule Hora University

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Education

January, 2009-October 2011, Swiss Tropical and Public Health Institute (University of Basel), Switzerland, PhD in Epidemioloy

September, 2007- January, 2009: Swiss Tropical and Public Health Institute (University of Basel), Switzerland, M.Sc in Infection Biology and Epidemiology

September, 2005- July, 2006: Institute of Tropical Medicine, Antwerp, Belgium, M. Sc, in Tropical Animal Health.

1993-1999: Addis Ababa University, Faculty of Veterinary Medicine, Ethiopia, DVM

1989-1990: Addis Ababa University, Faculty of Veterinary Medicine, Ethiopia, Animal Health Diploma.

1980-1988: From grade one up to grade twelve completed at Harakalo

Elementary School & Negelle Junior & Senior Secondary High School, Ethiopia

Thesis

1. Mycobacteria and zoonoses among pastoralists and their livestock in south-east Ethiopia, PhD thesis 2011, Swiss Tropical & Public Health Institute (Basel University), Switzerland
2. Prevalence of tuberculin reactors in pastoral cattle herds in the Oromia region, Southern Ethiopia, M. Sc thesis 2009, Swiss Tropical & Public Health Institute (Basel University), Switzerland
3. Molecular typing of multi-drug resistant *M. tuberculosis* isolates from Rwanda ,M. Sc thesis 2006 , Antwerp Belgium
4. Observation on diseases of one-humped camel: Abattoir survey in southern Ethiopia Borana , DVM thesis 1999, Addis Ababa University, Debrezeit, Ethiopia

Work experiences

1. **Current positions:**

As of January 2011 Vice president for Academic and Research at newly opened Bule Hora University, Ethiopia

2. **September 2003 –Sept, 2007:** Jimma University College of Agriculture & veterinary Medicine, Ethiopia

Duties: Teaching different veterinary medicine courses. In addition to teaching and researches engaged in other College activities such as Community Based Education program coordinator, academic commission member and Research & Publication Committee member

3. **Jan. 2000-August 2003:** Borana low lands Pastoral Development program/GTZ, Borana, Ethiopia

   Positions: a) Pastoral Livestock production & health co-coordinator

   b) A/Deputy team leader for six months.

   Duties

- Coordinate livestock disease control and prevention in collaboration with governmental and non-governmental institutions in the Southern pastoral areas

of Borana low lands.

- Facilitate research activities on animal health & production in the Borana lowlands.
- Facilitating participatory animal health & production development strategies (animal husbandry, veterinary services, marketing, and animal production diversification such as bee keeping, poultry, and riverine fisheries) considering the traditional knowledge and social structure.
- Coordinating the development, test, and promotion of a pastoral and agro pastoral orientated participatory extension concepts for the animal health & production sectors including training of extension staffs.
- Organizing and coordinating sectoral and inter-sectoral working groups.

4. **October 1990- October 1993:** Wondogenet College of Forestry, Ethiopia
   Position: Assistant veterinarian of college dairy farm

Publications and submitted manuscript

Gumi B., Firdessa R., Yamuah L., Sori T., Tolossa T., Aseffa A., Zinsstag J. & Schelling E. (2012) Sero-prevalence of brucellosis and Q-fever in South-East Ethiopian pastoral livestock. *Draft Manuscript.*

Firdessa R., Berg S., Hailu E., Schelling E., **Gumi B**., et al, (2012). Investigation of Pulmonary and Extrapulmonary Tuberculosis in Ethiopia shows Minimal Zoonotic Transmission of *M. bovis* and identifies a new Lineage of *M. tuberculosis.* *Manuscript submited to EID*

Gumi B., Schelling E., Berg S., Firdessa R., Erenso G., Mekonnen W., Hailu E., Melese E., Hussein J., Aseffa A., & Zinsstag J. (2012) Zoonotic transmission of tuberculosis between pastoralists and their livestock in South-East Ethiopia. *Ecohealth* 9 (2), 139-149

Gumi B., Schelling E., Firdessa R., Erenso G., Biffa D., Aseffa A., Tschopp R., Yamuah L., Young D., & Zinsstag J (2011). Low prevalence of bovine tuberculosis in Somali pastoral livestock, South-East Ethiopia. *Tropical Animal Health and Production,* DOI: 10.1007/s11250-012-0085-5

Gumi, B., Schelling, E, Firdessa, R, Aseffa, A, Tschopp, R, Yamuah, L, Young, D & Zinsstag, J (2011) Prevalence of bovine tuberculosis in pastoral cattle herds in the

Oromia region, southern Ethiopia. *Tropical Animal Health and Production*, 43 (6):1081-1087.

Markos T. & **Gumi B**. 2006. Evaluation of Bulla as solidifying agent for Microbiological Media *Ethiopian Veterinary Journal*. 10 (1), 87-97.

Woldemeskel, M.& **Gumi. B** 2001: Prevalence of Sarcocysts in One-humped Camel (*Camelus dromedaries*) from southern Ethiopia. *Journal of Veterinary Medicine B* 48, 223-226.

Woldemeskel.M. & **Gumi** B 2001. Study on Disease of one-humped Camels (*Camelus dromedaries*) in Borana Ethioipia. BULL. ANIM. HLTH.PRD.AFR, 49,179-182.

International conferences and Training attended during PhD study.

1. Keystone Symposium on Mycobacteria: Physiology, Metabolism and Pathogenesis - Back to the Basics, January 15- 20 2011, Vancouver, Canada

   Poster presentation: Tuberculosis in South-East Ethiopian Pastoralists and their livestock.
2. Conference of Research workers on Animal Diseases, December 5-7, 2010, Chicago, USA.

   Oral Presentation: Bovine Tuberculosis at the Livestock-Human interface in Pastoralists communities of Southern Ethiopia
3. Annual Conference of Swiss Society of Tropical Medicine and Parasitology October 27-29, 2010 Spiez, Switzerland.

   Oral presentation: Tuberculosis at the livestock-human interface in pastoralist communities of southern Ethiopia.
4. Integrative Training and Capitalizing on Experiences (ITC 2010), NCCR North-South, September 1-12, 2010, Bahir Dar, Ethiopia

   Poster and Oral presentations: Bovine tuberculosis and other zoonoses among pastoralists and their livestock, Ethiopia
5. Annual Conference of Swiss Society of Tropical Medicine and Parasitology November 25, 2009 Basel, Switzerland.

   Oral presentation: Epidemiology of mycobacterial infection in animals and humans in pastoral areas of southern Ethiopia

Awards/Fellowship

1. Bill and Melinda Gates Foundation Global Health Travel Award (2011)
2. Swiss Government foreign student Scholarship Program, Switzerland (2007-2010)
3. Wellcome Trust fund Bovine TB research program, AHRI/Ethiopia (2007-2011)
4. DGDC, Antwerp Belgium (2005-2006)

References

1. Prof. S. Geerts, E-mail: SGeerts@itg.be (M. Sc supervisor)
2. Dr. L.Rigouts, E-Mail: LRigouts@itg.be(M. Sc co-supervisor)
   Institute of Tropical Medicine, Nationalestraat 155-2000, Antwerp, Belgium.
3. Prof. Jakob Zinsstag, Email: Jakob.Zinsstag@unibas.ch (PhD supervisor)
4. Dr. Esther Schelling, E-mail:Esther.Schelling@unibas.ch (PhD co-supervisor)
   Swiss Tropical and Public Health Institute, P.O.Box, 4002 Basel, Switzerland
5. Dr. Abraham Aseffa (PhD co-supervisor), Armauer Hansen Research Institute, P.O.Box 1005, Addis Ababa Ethiopia